

Mushroom Production technology

Various levels of technologies are available for production of button mushroom- right from cottage industry of China to automated and mechanized technology of the developed countries. The present project proposes to adopt the modern technology of mushroom growing under controlled growing rooms with necessary mechanization and automation owing mainly due to large size of the project and handling of the raw materials in bulk on regular basis to achieve uniform and constant production. This shall cut down the cost of production and improve the quality of mushrooms. Low cost of production will boost competitiveness in the national and international market.

PRODUCTION TECHNOLOGY OF *AGARICUS BISPORUS*

Unlike other crops, cultivation of white button mushroom is a complex process and requires special technical skill for raising a successful crop. *A. bisporus* for its growth requires 22-28°C temperature for spawn run and 15-18°C for its fructification. Besides this it requires 85-90% RH and enough of ventilation. Due to low temperature requirement the cultivation is more popular in hilly region. However, due to advancement of the cultivation technology and advent of the controlled facilities its cultivation is now successfully extended to the plains. Cultivation of white button mushroom requires three basic steps

1. **Production or procurement of spawn**
2. **Preparation of selective medium (compost)**
3. **Production of Crop.**
4. **Processing**

1. SPAWN (MUSHROOM SEED) PRODUCTION:

In the first phase of implementation of the project, the spawn (seed) will be procured from a reliable source/ laboratory situated nearby or directly from ICAR-DMR, Solan (HP). In the second phase of increasing the capacity of the unit it will be produced in-house.

To get improved yields and quality latest hybrids like S -130, S- 140, A-15, NBS-5 etc. which give optimum production in 30 days of cropping will be used to ensure minimum 6-7 crops per room per year.

MUSHROOMS - CULTURE AND SPAWN

Mushroom cultivation for food and medicine is a well established profitable industry in many countries of the world. Nevertheless, strain improvement of cultivated mushrooms and the development of artificial cultivation of wild mushroom demand a well-planned system for the maintenance, preservation and availability of genetic diversity. Successful mushroom production depends upon the proper maintenance of pure culture spawn capable of providing higher yields, excellent flavour, palatable texture, colour and resistance to pests and diseases. Maintenance of vigour and genetic characteristics of a strain in form of a pure culture is very important. The isolation, purification and maintenance of mushroom cultures require technical expertise and aseptic high-tech laboratory facilities. Therefore, small mushroom growers can't maintain their own pure culture and/or spawn. They have to rely entirely on commercial spawn producers, reliable governmental or non-governmental organizations that play a vital role in supplying reliable spawn of the desired mushroom strain or variety. Hence training any established organization is a prerequisite before starting a spawn lab.

Culture isolation

In nature all types of mushroom use dead plant materials as a source of nutrients, which are made available through different degree of decomposition. The culture media or substrate for isolation and culturing must meet the nutritional requirement of mycelium. Some of the commonly used media are: Potato Dextrose Agar, Malt extract, Oat meal, agar, Compost agar and Wheat extract agar. These can be

used to isolate and multiply the mycelium of edible mushroom fungi in petridish/bottles/test tubes. A tissue from apex (rapidly growing cells) of the stipe or inner tissue of the cap is often used for obtaining the mycelial cultures. Tissue from other parts however, can also be used. Often 1 or 2 tissue cubes are placed per surface of replicated bottles or plates. These 'inoculated' bottles or plates are then incubated at room temperature (supra-optimal mycelial growing condition) until mycelium reaches to the required stage. In laboratory, the edible mushroom strains are cultured on various culture media such as:

Potato Dextrose Agar (PDA) Peeled potato - 200 g Agar agar - 20 g Dextrose - 20g Distilled water - 1 lit	Malt Extract Agar Malt Extract – 20 g Agar agar – 20 g Dextrose – 20 g Distilled water – 1 lit
Oat meal agar Oat meal flakes - 30 g Agar agar - 20 g Distilled water - 1 lit	Compost agar Pasteurized compost - 150 g Agar agar powder - 20 g Distilled water - 1 lit
Wheat extract agar Wheat grain - 32 g Agar agar powder - 20 g Distilled water - 1 Lit.	Rice bran decoction medium Rice bran - 200 g Agar agar - 20 g Distilled water - 1 Lit

Wheat grain and compost extract are most suitable culture media for maintaining *A. bisporus* and *A. bitorquis* cultures. Cultures of *Volvariella* spp. and *Pleurotus* spp. can be maintained on PDA or Malt extract agar medium. It is desirable that cultures are not maintained on the same type of culture medium.

Fresh and healthy mushroom fruit body (basidiocarp) showing all the desirable attributes of that species/strain should be used to raise mycelial cultures by the following methods:

(i) Vegetative mycelium culture (tissue culture)

Under aseptic conditions using laminar flow, young basidiocarp is cleaned with sterilized distilled water and dipped into 0.1% mercuric chloride or 2.5% sodium hypochlorite solution for 1 min. In case of button mushrooms the basidiocarp is air dried and split open longitudinally from centre and vegetative mycelial bits are cut from the collar region (junction of pileus and stipe). Whereas, in black ear mushrooms, the ear is cut along the edge with a sterilized scissor and inner tissues are scraped and small bits of tissues are removed. These bits are then placed in oven sterilize petriplates having culture media. Inoculated plates are incubated at $25 \pm 2^{\circ}\text{C}$ in a BOD incubator. Within 4-5 days the new mycelium growing from the tissue is observed. The pure cultures are made by carefully transferring young mycelium from growing edge of the colony from petriplate to test tubes and again incubating at $25\text{C}\pm 2^{\circ}\text{C}$ for 10-14 days (35°C for *Volvariella* spp.).

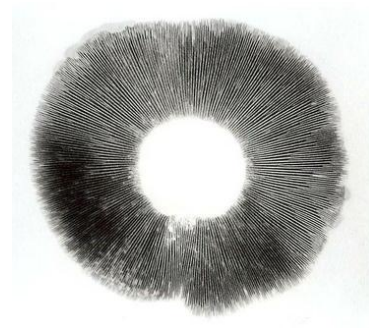


It is important to check the cultures from time to time for growth and contamination. Mycelial disc of appropriate agar block size are then transferred to new bottles or test tubes and used as the first multiplication of that culture line. Various workers have observed variation in tissue culture propagated strains and reported that majority of the tissue cultures isolates (TCIs) produced low yield in comparison to parental strains. The variation for yield was also revealed among TCIs raised from different parts of fruit bodies. Pileus cultures exhibited minimum variability and yielded better than cultures from stipe and gill tissues. Somaclonal variation can also arise during propagation of various strains via tissue culture

due to occurrence of somatic recombination, increased activities of transposons (jumping genes), mutation, gene frequency. The researchers use tissue cultures mainly for domestication of wild germplasm and maintenance of existing varieties.

(ii) Multispore culture

Under aseptic conditions, spore mass is scraped from a fresh spore print or basidiocarp and suspended in 100 ml of sterilized distilled water in flasks and shaken to obtain uniform spore suspension. A few drops of this suspension are added to lukewarm culture medium and poured into oven sterilize petriplates. Petriplates are rotated to homogenize the spore suspension into culture medium. The culture medium is allowed to solidify and then petriplates are inoculated at $25 \pm 2^\circ\text{C}$ for 3-4 days (35°C for *Volvariella volvacea*). The germinating spores are transferred carefully to culture tubes along with a piece of agar containing a culture medium recommended for the mushroom species being isolated. The culture tubes are then incubated at 25°C for 10-14 days in case of *Agaricus bisporus* and *A. bitorquis*, and at 32°C for *Volvariella volvacea* for 7 to 10 days.



(iii) Single spore culture

A. bitorquis and *Pleurotus* spp. are heterothallic with tetraspored basidia, therefore single spore are self sterile but this technique can be successfully used for breeding new strains. *A. bisporus* being secondary homothallic with bispored basidia and majority of its spore being self fertile, can be used to raise fertile cultures. Single spore cultures are procured in same way as that in multispore cultures. Nevertheless, for single spore culture isolation, the spore suspension is serially diluted to obtain 10-15 spores per plate so that individual germinating spore is marked and could be lifted and transferred to culture medium and incubated at $25 \pm 2^\circ\text{C}$ for 10-14 days. Cultures of edible mushrooms can be preserved either as spores or vegetative mycelia. The techniques for maintenance of mycelia for short, medium and long term are described below.



Culture maintenance

(i) Frequent sub-culturing

Under recommended temperature and pH conditions, most mushroom mycelium continues to grow till the nutrients of a suitable culture medium are exhausted. Therefore, these cultures remain viable only for few months depending upon the growth rate, substrate, method of storage, etc. Using a system of periodic transfer at reasonable intervals, stock cultures are often maintained in an actively growing state under optimum laboratory conditions. After obtaining optimum mycelial growth, mushroom cultures are stored until sub-culturing is necessary. For storage purposes cultures are prepared on agar slants in culture bottles and or test tubes. These cultures can be stored in racks at room temperatures for one to few weeks. The periods between sub culturing can be extended up to 4-6 months by storage at cooler temperatures, i.e., at $4-7^\circ\text{C}$ in a refrigerator or cold room.



In laboratory, the edible mushroom strains are subcultured on suitable culture media. *Volvariella volvacea* is incubated at 32°C for 7 to 10 days. The other mushroom strains are incubated at 25°C for 2-3 weeks until the slants are fully covered with mycelium. Once fully grown culture of *V. volvacea* has been obtained, they are to be kept at room temperature. *V. volvacea* should be subcultured every 2 months. Species of *Lentinula*, *Pleurotus* and *Agaricus* strains can be kept in a refrigerator at 4°C, and they should be subcultured every 6 months. Deviation from the original characteristics of the cultures can be detected with mycelial cultures. The most common degenerative symptoms are sectors of slow growth, mycelium that is thin and weak in appearance, or matted or fluffy but has normal growth rate. A slow growing mycelium needs more time for colonization and tends to carry virus particles. A fluffy mycelium causes the grain to stick together and is harder to spread in compost than normal grains. It tends to form stroma and it gives lower yields. Mycelia of these types should be discarded. Culture tubes of *Volvariella* spp. forms clamydospores, which are brownish in colour. Culture tubes showing more clamydospores indicates that the culture has a good vigour and will be high yielding type. Nevertheless, partial loss of mushroom forming capacity and the desired qualities because of degeneration and mutation during prolonged vegetative propagation of stock cultures, or from genetic recombination and selection in continuous field cultivation of re-established culture is relatively common in the spawn produced from cultures maintained by these methods. Furthermore, such conventional procedures of conservation of living fungi are time consuming, costly and risky. Ultimately repeated subculturing may result in preserving a culture different from the original one. The disadvantages of frequent sub culturing are loss of desirable traits, chances of contamination by air borne spores or mites carried infections, constant specialist supervision, labour intensive and time consuming process etc.

(ii) Storage under mineral oil

The mineral oil (Liquid Paraffin or Medical Paraffin specific gravity 0.830 – 0.890 g) is sterilized in an autoclave at 121°C for 15 minutes for two consecutive days. Short slants require less oil to cover them. Coverage must be complete as strands of mycelium left exposed may act as wicks to dry out the culture. Therefore, actively growing mycelial cultures are covered upto 1 cm above the slant level. Alternatively, 0.5 cm mycelial discs are suspended in 1-2 ml of sterilized liquid paraffin. Covering cultures on agar slants with mineral oil prevents dehydration, slows down metabolic activity and growth through reduced oxygen tension. The mineral oil blocks the exchange of oxygen between the mycelial surface and the atmosphere in the container, thus retards metabolism and also prevents desiccation of the agar medium. In conjunction with maintenance of the culture in a refrigerator at 4°C, this is an effective method of preserving fungal cultures. Retrieval is done by removing a small piece of the fungal colony or disc with a sterile needle, hook or loop, draining off as much oil as possible and streaking the inoculum onto agar in plates or tubes. Tilting the plate or bottle may facilitate drainage. The first subculture often has a reduced growth rate and a second culture is usually required before a good culture is obtained.



Two disadvantages of oil storage are contamination by airborne spore and retarded growth on retrieval. Nevertheless, in our laboratory, this method is working very well for conservation of most mushroom cultures satisfactorily for several years. A simple and economic method of long-term preservation of mushroom culture for tropical countries using *Pleurotus pulmonarius* involves taking 3 mm diameter discs from agar cultures of fungus in petridishes and storing the discs at room temperature in glass tubes (9 x 75 mm) containing 1 ml of liquid paraffin and plugged with non-absorbent cotton covered with tin foil. The culture stored in this way remained viable for 8 years.

(iii) Water storage

The cultures are grown on a suitable culture medium and after full growth, 4-5 bits of 0.5 mm diameter are transferred aseptically to pre-cooled and sterilized McCartney bottles containing simple water and the lids tightly screwed down and are stored at room temperature. All mushroom cultures except *V. volvacea* can be stored by this method. Demineralized water proved better. Revival of culture is by removal of a block and placing the mycelium on a suitable growth medium. Survival of fungal cultures stored this way is reported for 2 to 5 years period satisfactorily at IMI, Kew, Surrey, U.K. In another study water storage of 650 plant pathogens belonging to the Phycomycetes, Ascomycetes, Fungi Imperfecti and Basidiomycetes remained viable for 7 years. Growth may sometimes occur during storage in water. This can be reduced if the spores or hyphae are removed from the surface of agar without medium and transferred in water blanks.

(iv) Lyophilization

Lyophilization, also known as freeze-drying, is a method of choice for long-term Preservation of spore-bearing fungi. Mycelial mushroom cultures are not well preserved in this way. However, spore collected from a young and healthy mushroom aseptically can be stored for several years by this method. In freeze-drying, spore are frozen and water is removed by sublimation. The drying of the spores is accomplished by freezing under reduced pressure in a vacuum. Stability and long storage periods have been shown to be the main advantages of freeze drying.



Most commonly used suspending media for freeze-drying are skimmed milk (10%), or (trypticase soy broth (0.75 g) with sucrose (10 g) and Bovine-serum albumin (5.0 g) in 100 ml distilled water) which are used with equal volume of culture suspension. Freeze-drying of basidiospores of mushroom can be done by adopting following procedure of freeze-drying. The glass ampoules are first sterilized in a hot air oven at 130°C for 2-3 hours and are plugged with cotton. These ampoules are autoclaved for 15-20 min. at 121°C at 15 lb psi. Culture suspension in case of mushroom or spore suspension in other fungi is prepared in skim milk or suitable medium. Each sterilized ampoule is then filled with 0.2 ml of culture suspension. A few aliquots are serially diluted to determine pre-freezing viable count. Rest all the ampoules with spore suspension are placed in a freezer (-70°C) for 1 to 2 hours. When shelf temperature of the freeze chamber reaches -40°C, ampoules with frozen samples are placed inside the chamber of freeze-dryer (Lyophilizer) and vacuum is created. Primary drying is achieved at -40°C for 4 hours. Temperature is then raised in 10°C increments keeping at each temperature for at least half an hour and at 20°C for one hour. Vacuum is released and ampoules are stored at -20°C (or -70°C). Next morning samples are dried at least for 2 hours and vacuum released. Cotton plugs are then pushed inside down and constrictions are made in the ampoules above the cotton plug. The ampoules are then attached to the freeze-dryer for secondary drying under vacuum at 20°C for 2 hours and sealed while attached to the lyophilizer itself with the help of a gas-air torch. The ampoules are then stored at 4°C to 6°C for longer shelf life inside a refrigerator. A representative ampoule can be cut from the top to check post-freezing count before finally storing the ampoules for longer duration. Viability of most organisms does not change much upon freeze-drying of viable spores. Rehydration of the fungi with sterile distilled water should be carried out slowly for 30 min. for absorption of moisture before plating on a suitable culture medium.

It has been demonstrated that hyphal cooling at the rate of -1°C/minute to temperatures of -45°C and then -75°C, produced fully freeze-dried mycelia. Freeze drying was performed for 2 hours at -40°C followed by 20 h at -2°C and 8 hours at 20°C, resulting in a residual moisture content of 2%. Hyphae of Ascomycetes as well as Basidiomycetes survived freeze-drying.

(v) Preservation at -70°C

Glycerol (10%) in aqueous solution is sterilized by autoclaving at 121°C for 15 minutes. Alternatively Dimethyl sulfoxide (DMSO) is sterilized by filtration using 0.22 micron Teflon filter. Usually 10% glycerol suspension of cultures is made (0.5 ml to 1 ml) and the aliquots are distributed in small vials or tubes. The vials/tubes are placed at -70°C . DMSO penetrates more rapidly and is often more satisfactory and may also be used as cryoprotectant in place of glycerol. Many culture banks are maintaining mushroom cultures by this method satisfactorily for several years (e.g. Microbial Type Culture Collection, Chandigarh).

(vi) Cryopreservation in liquid nitrogen

The storage of micro-organism at ultra low temperatures (-196°C in liquid nitrogen) is at present regarded as the best method of cryopreservation. Lowering the temperature of living cells reduces the rate of metabolism until, when all internal water is frozen, no further biochemical reaction occurs and metabolism is suspended. Although little metabolic activity takes places below -70°C , recrystallization of ice or ice crystal growth can occur at temperature above -139°C , and this can cause damage during storage. The volume occupied by water increases by 10% when water crystallizes and form ice. This puts the cell under mechanical stress. At -196°C dormancy is induced, during which the organism does not undergo and change either phenotypically or genotypically, provided adequate care is taken during freezing and thawing. This method can be applied to both sporulating and non-sporulating cultures. Optimization of the technique for individual strain has enabled the preservation of organisms that have previously failed.



The temperature of liquid phase of nitrogen remains at -196°C and average temperature of the vapour phase is around -140°C . Glycerol (10%) suspension of young mushroom mycelium is prepared and distributed in aliquots of 0.5 ml to 1 ml in plastic screw cap cryo-vials, which can withstand ultra cold temperature. At some culture banks 0.5 mm disc are suspended in 10% glycerol solution. Programmed cooling at 1°C to 10°C per minute is ideal. In case where programmable freezer is not available, vials are first placed in a mechanical freezer (-70°C) for an hour and then to check viability of a culture before and after freezing. Glycerol may be replaced by 5% DMSO. Cultures may be recovered by rapid thawing at 37°C . Presence of liquid nitrogen in storage vials may cause explosion while thawing.

It has been reported that culture viability and mushroom production were not affected by cryogenic storage for 9 years. In a study in the Glasshouse Crops Research Institutes, U.K. where 1,012 cultures of *Agaricus bisporus* and related species for 3 to 4 years were stored and reported 95% recovery rate. Researchers found that 10% aqueous glycerol solution used as cryoprotectant was good in preserving the cultures of *Agaricus* spp., *Coprinus* spp., *Lentinula* spp., *Pleurotus* spp., *Schizophyllum commune*, *Tremella* spp., *Polyporus* spp., and *Volvariella bombycina* but not suitable for *Volvariella volvacea*. However, it was found that a 10% aqueous DMSO (dimethyl-sulfoxide) solution gave constant and reliable retrieval of *V. volvacea*.

(vii) Cryopreservation in mechanical freezers

Because viability of stored cells increases dramatically with lower temperature, the ultra low temperature mechanical freezers are recently designed by leading multinational companies to operate efficiently at -140°C or -150°C . Cells may be stored indefinitely at sufficiently low temperatures, safely below -130°C , which is glass transition temperature of water. Below this temperature enzyme activity is completely suspended and thermally driven reaction cannot occur. The cultures are prepared in the same way as for liquid nitrogen preservation and placed first at -20°C and then at -70°C and finally in freezers

maintained below -130°C . The culture preservation by this method is as good as in liquid nitrogen. It is cost effective when compared to the cost of per litre refill charges of liquid nitrogen, thus reducing operating expenses. Nevertheless, ultra low temperature freezers are run on electricity and therefore are not very successful in developing countries where electricity supply is erratic and on the spot repairs are inaccessible.

It is recommended that each mushroom strain/isolate should be maintained by at least two different methods. In general, storage in liquid nitrogen and mineral oil preservation technique are best suited for preservation of edible mushrooms. The handling techniques, freezing protocols, cryopreservation and thawing rates can be optimized for a particular strain to obtain maximum survival. Once the mushroom has been successfully frozen and stored in liquid nitrogen, the storage period appears to be indefinite, because no chemical and or physical changes can occur at such low temperatures.

Culture Multiplication as Spawn

(i) History and types of spawn

The term spawn has been defined as the vegetative mycelium from a selected mushroom grown on a convenient medium. The spawn comprises mycelium of the mushroom and a supporting medium, which provides nutrition to the fungus during its growth. Culpeper described spawn as mycelium of mushroom. From 1652 to 1894 A.D. spawn was gathered from the wild rather than made. Before the advent of grain spawn in 1932, different kinds of spawn used were natural or Virgin spawn (from the pastures & meadows), Flake spawn (breaking of beds through which mushroom mycelium has run), Mill-track spawn (bricks dried and made from mixture of horse dung, cow dung and loan soil), etc. In the beginning of 20th century pure mycelial culture were made and used for making manure spawn on sterilized horse manure or compost manure.

The first pure culture spawn was produced by Contratin in France (1894) on horse manure compost. In 1905 Duggar prepared pure culture from mushroom tissue. Later on spawn were used to inoculate sterilized horse manure in bottles (1918). The process of making spawn on grain was introduced by the Pennsylvania State University, which held two patents on it. These patents were assigned to the university by the inventor, Professor J.W. Sinden in 1932. Licenses under the patent were available to any laboratory qualified to make the grain spawn. Grain spawn had an advantage over manure spawn as it could be mixed easily and provided many inoculum points. The grain spawn was further perfected by Stoller in 1962. Since the process for the production of grain spawn, the fundamentals have not changed. You still need a starter culture, cereal grain, the grain is sterilized, cooled and the product grown out. It is no secret that anyone can make spawn, just as anyone can grow mushrooms. Today most of the traditional spawn laboratories world over are using wheat, rye and millet grains as substrate for spawn production and are following the standard technique of mother spawn from pure culture mycelium grown on synthetic medium.

At present, the pure culture spawn has been the basis of modern spawn production units all over the world. The manufacture of the pure culture spawn is done under scientifically controlled conditions which demand a standard of hygiene as in a hospital operation theatre. Equipment and substrate used for spawn are autoclaved and filtered air is passed during the inoculation ensures complete freedom from contamination.



(a) Manure spawn

Either composted horse-dung or synthetic compost may be used. The composted manure is thoroughly washed to remove such substance in compost which retards growth. The excess water is squeezed out and moisture content adjusted to 60%. The manure is packed in half-litre milk bottles or heat-resistant polypropylene bags of suitable size. The bottles or bags plugged with non-absorbant cotton-wool/poly-fill and sterilized in an autoclave at 121°C for 2 hr or on 2 consecutive days for an hour each. They are then inoculated with a large bit of agar-containing mycelium and incubated at 22-24°C in a dark place. the spawn can be used to inoculate fresh bottles or bags to obtain the second generation spawn.



(b) Grain spawn

Ten kilograms of wheat grains are boiled for 15 min in 15 litres of water and then allowed to soak for another 15 min without heating. The excess water is drained off and the grains are cooled in sieves. Turn the grains several times with a spoon for quick cooling. The cooled grains are mixed with calcium carbonate. The gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and 30 g of calcium carbonate. The gypsum prevents the grains from sticking together and calcium carbonate is necessary to correct the pH. The prepared grains are filled into half-litre milk bottles or polypropylene bags (at the rate of 150-200 g per bottle or bag) and autoclaved for 2 hr at 121°C. After sterilization, the material should have a pH value of 6.5 to 6.7. The bottles are inoculated with grains spawn or with bits of agar medium colonized with mycelium and incubated at 22-24°C in a dark place. the mycelium completely permeates the grains in about 2 weeks. Other grains like sorghum and pearl millet can also be used for spawn making.



(c) Perlite spawn

This was developed by Lemke (1971). Perlite is a mineral which expands at temperature more than 1000°C. The ingredients of the spawn are: Perlite (1,450 g), wheat-bran (1,650 g), gypsum (200 g), calcium carbonate (50 g), and water (665 cc). The ingredients are mixed, filled in bottles and sterilized. Thereafter, the process is the same as for grain spawn. Perlite spawn is easy to disperse and can be produced at a cheaper cost. This spawn can be stored for a long time.

(d) Saw dust spawn

Sawdust spawn is a sterilized mixture of sawdust and bran fully colonized with mushroom mycelium. This kind of spawn is normally used for the cultivation of wood rotting mushrooms like Shitake, Reishi, maitake, etc.



(e) Liquid spawn

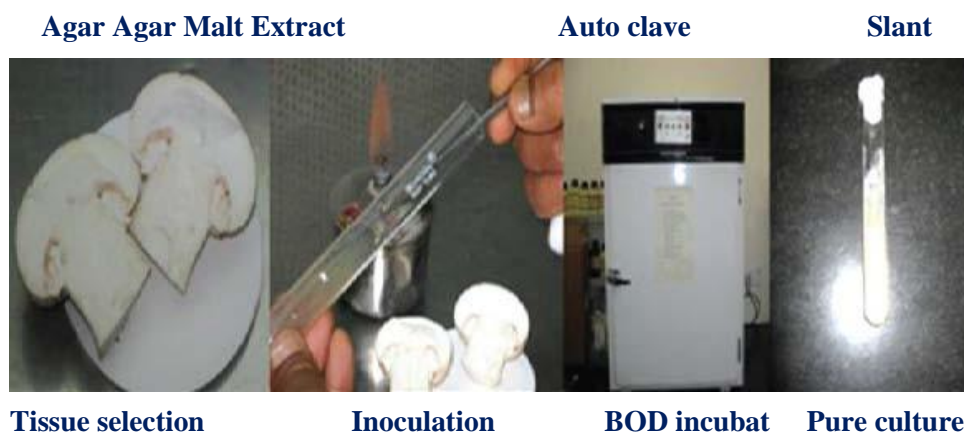
Mycelium cultured in liquid medium followed by maceration/ homogenization can also be used for spawning. This is commonly referred as liquid spawn. It can be used for mechanizing inoculation process of spawn multiplication or can be used for inoculating substrates.

Today most of the traditional spawn laboratories world over are using wheat, rye and millet grains as substrate for spawn making and are following the standard techniques of mother spawn from pure culture mycelium grown on synthetic medium. Strandy cultures showing good growth and not showing fluffy growth, sectoring or slow growth are desirable. During cropping bare patches on bed, deformed fruit bodies with no or few gills, weeping mushrooms indicate strain degeneration. Multispore cultures degenerate faster than single spore cultures. Hence, it is important to properly maintain the culture at desired temperature and rejuvenate them by change of media and replace them in case of any sign of degeneration.

Pure Culture Preparation

Pure culture of fleshy fungi/mushrooms can be prepared either by multi-spore culture or tissue culture. Multi-spore culture is obtained by placing a fresh fruit body after alcohol sterilization on a petriplate/sterilized paper. Millions of spores are collected within 48hr. Serially diluted loop full of spores are then transferred to sterile Potato-dextrose-agar (PDA) or Malt-extract-agar culture slants. These slants are then inoculated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 2 weeks to obtain pure culture. For tissue culture, the basidiocarp after alcohol sterilization is cut longitudinally into 2 halves and bits from collar region are transferred to pre sterilized PDA or MEA culture medium. The petriplates are incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in BOD incubator for one week. Mycelium from growing edges is carefully transferred to MEA/PDA slants and again incubated for 2-3 weeks to obtain pure cultures. Basic materials and equipment required for obtaining pure culture is given in Fig. that follows.





(ii) Substrate Preparation

Mushroom spawn can be prepared on any kind of cereal grains like wheat, jowar, bajra or rye and agricultural waste like corn cobs, wooden sticks, rice straw, saw dust and used tea leaf etc. Spawn substrate should have following desirable characteristics.

- i) It should not contain any inhibitory compounds to desirable mushroom species.
- ii) Large surface area of substrate should be available for fungal colonization.
- iii) It should provide essential nutrients required by mushroom mycelium to grow.
- iv) Cereal grains should be free from diseases.
- v) Cereal grains should not be broken, old, and damaged by insect pests.

Most of the cereal grains are good substrate for spawn production of white button mushroom (*Agaricus bisporus* and *A. bitorquis*), oyster mushroom (*Pleurotus* spp.) and paddy straw mushroom (*Volvariella volvacea*, but wood rotting fungi like shiitake (*Lentinula edodes*) and black ear mushroom (*Auricularia* spp.) grow better on saw dust based substrates over cereal grains. The grains are thoroughly washed in sufficient water three to four times to remove soil debris, straw particles and undesirable seed of grasses etc. washed grains are then soaked in sufficient water for 20-30 minutes and boiled in some container for 20-25 minutes. Normally for soaking and boiling 20kg of wheat grain, 35 litres of water is required. Excess water from the boiled grains is removed by spreading on sieve made of fine wire mesh or muslin cloth. The grains are allowed to leave as such for few hours so that the water on surface is evaporated. Now the grains are mixed with Gypsum (Calcium sulphate) and chalk powder (Calcium carbonate) so that the pH of the grains is around 7 to 7.8 and they do not form clumps. Different people have given different ratios for mixing Gypsum and Calcium carbonate. The best results have been obtained by using 200g Gypsum and 50g chalk powder for 10kg grains (dry weight basis). The quantity of Gypsum and chalk powder influences their ratio for mixing. First Gypsum and chalk powder are separately mixed and then they are thoroughly mixed with the grains. This mixing should be done on a smooth surface after wearing gloves to avoid contamination. For commercial spawn production rotating drums can be used for uniform mixing.



(a) Boiling, sieving, autoclaving and inoculation



(b) Method of inserting ring, folding bag and plugging



(c) Preparation of master spawn and commercial spawn

(iii) Mother Spawn Preparation

About 300g prepared substrate is filled in glucose/milk bottles up to 2/3 volume and plugged with non-absorbent cotton. These bottles are then autoclaved at 22 lb p.s.i. pressure at 126°C for 1.5 to 2 hr. These autoclaved bottles are left in the room for 24 hours so that they are cooled and kept on laminar flow under U.V. tube for 20-30 minutes. A piece of growing mycelium is aseptically transferred to these bottles and inoculated bottles are incubated at 25°C. Inoculated bottles are gently shaken on 5th and 10th day. Fully grain colonized mother spawn bottles can be used for inoculating commercial spawn bags after two to three weeks. For colonization these bottles are incubated at 22-25°C for *Agaricus bisporus*, *Pleurotus* spp. and *Lentinula edodes* but at 30°C for *Volvariella* spp.



Autoclave for sterilization of grains



Laminar Flow - Inoculation Chamber

(iv) Commercial Spawn Preparation

Commercial spawn can be prepared in polypropylene bags (heat resistant). Normally for half and one kg spawn the bags should be of 35x17.5cm and 40x20cm size, respectively. Polypropylene bags should have double sealing at the bottom and after filling the grains they are plugged with the help of a PP neck and non-absorbent cotton. The bags are then sterilized at 22lb p.s.i. pressure for 1.5 to 2 hours. Autoclaved bags are shaken well before inoculation so that the water droplets accumulated inside the bags is being absorbed by the grains. The sterilized bags are kept on the laminar flow under U.V. tube for 20-30 minutes. Ten to fifteen gm of grains from master spawn bottle is inoculated per bottle under aseptic condition or one bottle of master spawn is sufficient for inoculating 25 to 30 commercial spawn bags.



Inoculated bags are again shaken so that the inoculum is well mixed with other grains. Then the bags are kept in incubation room for mycelium spread. During incubation the bags are regularly examined for mould infestation. Contaminated bags should be immediately removed before discarding the bags to avoid build-up of contamination in the vicinity. Normally it takes 15-20 days for complete spread of mycelium on the grains. Fully colonized bags should be kept in cold room (+4°C) for future use. The spawn of button mushroom, *Pleurotus* can be stored at this temperature. However, neither the culture nor spawn of *Volvariella*, *Ganoderma* and *Calocybe* is stored below 15°C (Table). The contaminated bottles/ bags/tubes etc. are autoclaved before emptying and discarding.



Spawn Incubation

Table. Temperature requirement and storage and incubation of different mushrooms

	<i>Agaricus</i>	<i>Pleurotus</i>	<i>Lentinula</i>	<i>Volvariella</i>	<i>Calocybe</i>
Days for complete colonization of mother spawn	20-21	8-12	20-22	6-7	15-17
Days for complete colonization in commercial spawn	12-14	8-10	15-16	5-6	12-14
Incubation temperature (°C) during colonization	25	25	25	32	25
Storage temperature (°C)	4	4	4	15	15-16
Shelf life of spawn	Two months	One month	Three months	< 15 days	15 days

(v) Spawn Storage and its Transport

Wherever possible, freshly prepared spawn should be used because the mycelium is in the state of active growth. The spawn bags after completion of log growth phase are maintained of 2-3 months. The planting spawn should be systematically packed. It is transported for long distances during night so that the temperature of planter spawn does not rise beyond 30-32°C.

Scores of improvements have been reported in various step involved in spawn production. Earlier spawn was prepared in milk or glucose bottles, which was difficult to transport from one place to another. Heat resistant polypropylene bags have revolutionized the spawn industry. High-tech spawn labs now use

polypropylene bags with microfilm windows for aeration. Though polypropylene translucent bottles of 5-10 litre capacity are also used in Europe and USA for spawn production, but it has not been introduced in India due to high cost of the material. The mature spawn bags, that is polypropylene bags with grains fully colonized by mycelium should be packed in well ventilated cardboard cartons and stored at 2-4°C. The spawn is transported from one place to another in refrigerated vans or during night when temperature does not rise above 32°C. It is important that spawn bags are not exposed to heat and dust during transport.

Precautions

1. Always keep the inoculation chamber and its surroundings very clean.
2. Switch on UV tube in the inoculation chamber for 30 minutes before inoculation by keeping sterilized substrate, forceps, cultures inside the chamber
3. Inoculation is always done near the spirit the spirit lamp flame to avoid contamination.
4. The working person should swab his hands and inoculation chamber using alcohol.
5. Spawn should grow fast in the bottles, should be silky white in colour and should never show fluffy growth.
6. All grains should be covered by the mycelial growth and fresh spawn should have mushroom odour.
7. Mother spawn should not be used beyond 3-4 generations as it starts degeneration. Fresh spawn gives higher yield, therefore spawn should never be stored for more than a month.
8. All the bottles must be labeled indicating firms name, species, date of inoculation to know the age and type of spawn.

Characteristics of good spawn

Quality of spawn is mainly determined by the biological value of the strain used and technology involved at different stages of spawn production. The spawn should be fast growing in the compost, early cropping after casing, high yielding and should produce better quality mushrooms. Mushroom growers can not guess or predict the quality of spawn is being supplied to them unless the crop is grown from spawn. So it is the duty and responsibility of the spawn producing laboratories to stick to the schedule and standard of the quality spawn. There are few under mentioned characteristics of good quality spawn which can be judged by visual observation of spawn: Spawn prepared on Jowar or wheat grains gives higher yield over spawn prepared on bajra, barley or kodo grains.

1. There should be proper coating of mycelium around grains used as substrate for spawn production. No loose grain should be visible in a bottles/bags. The grains left over without mycelial coating will invite contamination in the compost during spawn running period.
2. The growth of the mycelium in the spawn bags/bottles should be silky/strandy type. It should not be cottony type because there is likelihood of stroma formation on the casing layer, which interferes with air exchange and absorption of water in the casing material resulting in low yields.
3. The growth of fresh spawn is more or less white. Brown colouration develops as spawn gets older. Fresh spawn gives higher yield than the old one. Spawn should not be more than one month old in any case.
4. There should not be any slimy growth in spawn bags/bottles which is an indication of bacterial contamination.
5. There should not be any greenish or blackish spot in the spawn bottles/bags. Such spots indicate that the spawn is contaminated with moulds.
6. When the spawn bags/bottles are opened for spawning it should emit typical mushroom smell.

Spawn Production (Flow chart)

Preparation of Mother Spawn

- Step-1 Select healthy and cleaned cereal grains
- ↓
- Step-2 Boil Grains in water (15-20 min.)
- ↓
- Step-3 Remove excess water on sieve
- ↓
- Step-4 Dry grains in shade (4 hrs.)
- ↓
- Step-5 Mix CaCO_3 (0.5%) and CaSO_4 (2%) on dry wt basis
- ↓
- Step-6 Fill 300g grains in glucose/milk bottle
- ↓
- Step-7 Plug cotton and autoclave at 22 p.s.i. for 1.5 to 2 hrs.
- ↓
- Step-8 Inoculate growing mycelium of desired strain using laminar flow
- ↓
- Step-9 Incubate in BOD at $23 \pm 2^\circ\text{C}$ for 20-25 days
- ↓
- Step-10 Master spawn is ready

Preparation of Commercial spawn

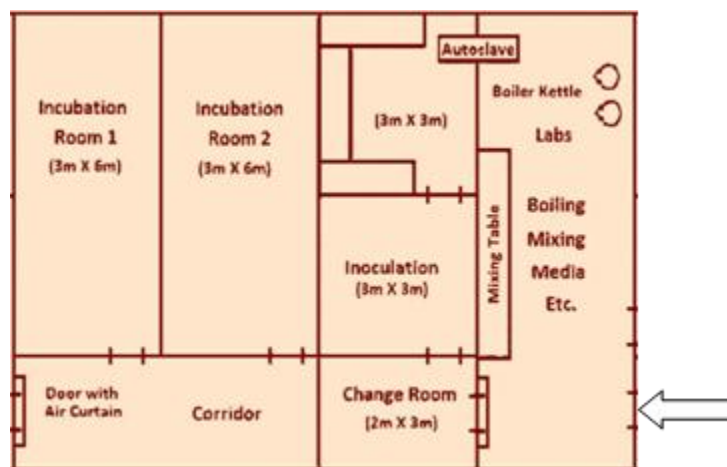
- Step-1 Use polypropylene bags instead of bottle
- ↓
- Step-2 Upto autoclaving (Step 1 to 7) is same as of mother spawn
- ↓
- Step-8 Inoculate 15-20 grains from mother spawn per PP bag
- ↓
- Step-9 Incubate at $23 \pm 2^\circ\text{C}$ in incubation room
- ↓
- Step-10 Shake bags after 7-8 days
- ↓
- Step-11 Commercial spawn is ready in 20-22 days.

Table. Problems faced, causes and solution during preparation of pure culture/spawn

Problem	Cause	Solution
Agar medium very soft or hardly solidifies	Quantity of agar insufficient i.e. too low or agar is of inferior quality	Use branded and proper quantity of agar in medium
Agar surface in the plates not smooth or lumpy	Agar medium partially solid when poured	Pour agar medium when it is still hot
Contaminants appear after 2-3 days on the surface of the medium after sterilization and before inoculation	Medium not sufficiently sterilized Medium not aseptically poured	Sterilization should be carried for the recommended period and at recommended temperature pressure Medium should be poured aseptically
Transferred mycelial bit/tissue resume no growth	Non-viable inoculum/culture Wrong type of medium Incorrect formulation or pH Needle or scalpel used to transfer the culture bit too hot or culture exposed to flame for prolonged period during transfer	Use viable culture/ actively growing culture Use correct medium Properly check the formulation and pH of the medium Cool the flamed needle before picking the inoculum and carefully transfer it
Contamination develops on the plugs after 2-3 days	Cotton too moist or not of non-absorbent quality Filters of the laminar flow damaged Incubation room too much loaded with air born inoculum	Properly cover plugs before autoclaving and use quality non absorbent cotton Filters should be checked or replaced as per recommendation Sterilize incubation rooms from time to time
Resulting mycelial growth slow and fluffy	Strain degenerated	Obtain another culture or retrieve stock culture
Grains contaminated after sterilization and before inoculation	Highly infected seeds Grains not fully sterilized Sealing of PP bags improper or plugs too loose	Use fresh and clean seed Prolong sterilization period Use quality PP bags (may use double bags) and properly plug the bottles/bags
Mycelial growth very thin and hardly penetrates the grains	Grains too dry	Boil the grains sufficiently and adjust proper moisture levels
Mycelial growth does not continue upto the bottom of the bag	Excessive grain moisture	Adjust proper moisture level
Mycelia do not grow thoroughly on the substrate or patchy growth	Grains contaminated with bacteria / yeast due to improper sterilization Less vigorous strain	Use recommended sterilization time Use vigorous strain
Contamination appears on the surface of the inoculum (grain or mycelia bit)	Contamination occurred during inoculation	Inoculation should be performed in a more aseptic way and observe complete cleanliness
Mycelia growing very slowly	Unsuitable substrate or processing improper, pH not correct, poor quality of gypsum or chalk Incubation temperature not suitable	Use recommended substrate Check the temperature requirement Use vigorous culture

G. Design of a Spawn Laboratory (Production Capacity 100 TPA)

The medium size spawn laboratory (production capacity at least 100 TPA) should have a total built up area of 96 x 8 x 3.2 m (L x B x H). This area will be divided into different work areas like cooking/autoclaving room, inoculation room, and incubation room, washing area. Cold storage room of 3 x 3 x 3.2 m (L x B x H) can also be added and it is enough to store the spawn at 4-5°C. The walls, roof floor as well as door is provided with heavy insulation (7.5-10 cm thickness) and two air conditioner (each of 1.5 tonnes capacity) are required to maintain temperature inside the room. Two incubation rooms of 3 x 6.0 x 3.2 m (L x B x H) with entire surface area (wall, floor, ceiling, doors) insulated with 5-7.5 cm thick insulation. Two air conditioners (each 1.5 tonnes capacity) are required to maintain temperature (25°C) in the incubation room.



H. Equipments Required

The equipment required in a spawn laboratory are:

1. Boiling pans/boiling kettle (vessel) for boiling the grains. Kettle can also be used if baby boiler is available otherwise kettles working on electricity, kerosene or gas can be installed. Pans for preparation of medium are also required.
2. Stove or steam line for boiling of wheat grains and preparation of medium.
3. pH meter to check pH of the medium.
4. Autoclave for sterilization of spawn medium and oven for sterilization glassware. Two electrically operated autoclaves with 100-145 bottle capacity having a dia. of 2 ½' and 3 ¼' height are sufficient. If boiler is available steam operated autoclaves can be used for better efficiency. A small clinical autoclave can also be kept for sterilization of culture medium.
5. BOD incubator is needed to incubate cultures and master cultures.
6. Laminar flow cabinet (normally 4 ft. horizontal) is needed for isolation and multiplication of cultures and spawns inoculation.
7. Refrigerator is needed for short-term preservation of mycelial cultures.
8. Other items like glassware, chemicals for medium preparation, non-absorbent cotton, polypropylene bags (or bottles), disinfectant (formaldehyde), calcium carbonate, calcium sulphate are also required.
9. Steel racks in incubation room and cold storage for keeping bags/bottles, exhaust fans, filters, office table, working tables, troughs, sieves, inoculating needles, scalpels, test tubes, petri plates etc. are also required.
10. AHUs can be installed for creating positive pressure by filtered air. Similarly air curtains are desirable to keep aseptic conditions in the lab.



Varieties/cultivars

Button Mushroom

Button Mushroom				
Varieties/ hybrids	Yield(kg)/100kg compost in 6- 8 weeks of cropping		Originating Breeder/Institute	Important quality traits
	Unpasteurized compost	Pasteurized compost		
A) <i>Agaricus bisporus</i>				
Varieties				
S-11	10-14	15-18	Mental, Germany	Long stiped, mushrooms open quickly, dark brown gills, widely adopted, good yield on LMC
S-791	8-9	14-16	Darlington, UK	Large and stout fruitbodies more dry weight, pink to light brown gills, high yielding.
MS-39	6-8	12-14	Single spore selection	Medium sized fruitbodies, light brown gills
P-1	6-8	14-16	Poona Selection	Long stiped, fruitbodies open quickly, dark brown gills
NCS-100	8-10	13-16	NRCM, Solan	Fruitbodies tough, short stiped, peak yield in first flush
NCS-101	8-10	14-16	NRCM, Solan	Fruitbodies tough with good post harvest qualities, peak yield in second flush
DMR-button-03	--	20-22	ICAR-DMR, Solan	Fruitbodies round cap with good post harvest qualities, peak yield in first flush

DMR-brown button-06	--	24-25	ICAR-DMR, Solan	Brown button, High yielding, very good quality fruit body.
S-454	--	16-18	Somycel	Good quality big fruit bodies, average wt 14-15 g
Hybrids				
U-1	-	14-18	Fritsche, holland	Suitable for canning, nice white medium sized fruit bodies
U-3 (SSI of U-1)	-	16-20	Fritsche, holland	High yielding, suitable for fresh marketing, strip broad and small, suitable for canning
NCH-102	-	16-19	ICAR-DMR, Solan	High yielding, uniform medium sized fruit body
NBS-1	--	21-22	ICAR-DMR, Solan	Fruit body White to off white, rounded and non-browning
NBS-5	--	23-25	ICAR-DMR, Solan	High yielding, Fruit body White to off white, rounded and non-browning
B) Agaricus bitorquis				
NCB-6 (RAB-50)	-	12-14	Vedder, USA	Medium sized white mushroom, more dry weight, good yielding
NCB-13 (PDAB-55)	-	13-15	Vedder, USA	Large extra white fruitbodies, more dry weight, high yielding
Hybrids				
K-26	-	13-15	Fritsche, Holland	Suitable for canning, good quality, high yielding
K -32	-	13-15	Fritsche, Holland	Long, stiped, easier to grow, more dry weight.

Productivity and suitability of different species/strains of oyster mushroom available for cultivation in India

Sr. no.	season	Species/strain	Yield g/kg dry substrate
A)	Winter season	<i>Pleurotus florida</i> strain-III	500-800
		<i>P.ostreatus</i> strain-III	500-600
		<i>P.ostreatus</i> strain-IV	600-800
		<i>P. eryngii</i>	400-700
B)	Summer season	<i>P.sajor caju</i>	600-700
		<i>P. florida</i>	600-700
		<i>P.sapidus</i>	500-600
		<i>P.djamor</i>	500-600
		<i>P.membranaceus</i>	500-600
		<i>P.cornucopicae-starin</i> -II	400-500

2. PREPARATION OF SELECTIVE MEDIUM (COMPOST):

Like other fungi *Agaricus bisporus* is a heterotrophic organism. It required carbon compounds that have already been formed by green plants. Besides carbon it requires nitrogen, essential elements such as phosphorus, sulfur, potassium and iron vitamins such as thiamine and biotin. All the ingredients that contain these compounds when fermented in a set pattern form a substrate, which is very selective in nature. On this selective substrate *A. bisporus* mycelium grows successfully at the practical exclusion of other competing micro-organisms.

About Raw Materials and Formulations of Compost

White button mushroom requires a well composted substrate for its growth. It is a saprophytic fungus and requires carbon compounds, which generally come from the agricultural waste materials. Besides carbon, it requires nitrogen and other essential elements, such as phosphorous, sulfur, potassium and iron, vitamins such as thiamine, biotin, etc. All the raw materials that contain these compounds are mixed in a fixed proportion and fermented in a set pattern to form a substrate, which is known as compost.

A. Raw Material and Ingredients

I. Agricultural base materials

These base materials form the bulk of compost and for this purpose wheat straw is favoured all over the world. However, quality compost can be prepared using variety of other materials including paddy straw, hay, barley, oat, maize stalks and leaves, sugarcane bagasse, sugarcane trashes and leaves, soybean stalks, mustard stalks, etc. These materials should preferably be freshly harvested / procured and should be around 5-8 cm long. These base materials act as a reservoir of cellulose, hemi-cellulose and lignin, which is utilized by button mushroom (*Agaricus bisporus*) during its growth as a carbon source. They also provide a little quantity of nitrogen. Besides acting as a nutrient source, they also add bulk to the compost, impart proper physical structure to the substrate and ensure adequate aeration during composting for the buildup of microflora essential for the composting process and also for the nutrition of mushroom. Rice and barley straw are very soft and degrade quickly during composting. These materials also absorb more water as compared to wheat straw. While using these materials care must be taken regarding quantity of water used for wetting, schedule of turnings and adjustment to the rate and type of supplements.

II. Supplements

Above base materials do not have adequate amount of nitrogen and other nutrients required to start the fermentation process having required C/N ratio. Also the requirement of nitrogen cannot be met with the little nitrogen available in straw. The compounding mixture is supplemented with other materials having nitrogen and carbohydrate sources. These materials can be classified as follows.

a. Animal manure

Horse manure undoubtedly is the best material for compost preparation. However, due to difficulties encountered in procuring good quality horse manure, use of this material has been restricted to few farms only. More and more farms are switching over to easily accessible materials. Chicken manure has proved to be the best alternative of horse manure. Other manures viz., pig, cattle and sheep have also been tried for compost preparation but with limited success. All these manures provide nitrogen to the compounding mixture, little of carbohydrate is also provided. These materials are highly variable in composition and their N-content may vary from 1 - 5 percent and it is released slowly during composting process. In addition to providing nutrients, they greatly increase bulk of compost, which is very important factor under Indian conditions considering the cost of wheat straw and these materials (specially chicken and horse manure). If horse manure is used in composting then it should be used along with bedding and urine, as it will not require any further supplementation. If it is not having enough bedding and urine when collected from a clean stable, supplementation with inorganic nitrogen along with some wheat straw may prove useful. Chicken manure if used, should preferably be a deep litter chicken manure having nitrogen content above 3%. If such manure is not available then the manure from cages can also be tried.

Chicken manure is generally used under short method of composting. However, some of the growers are using this under long method and are getting fairly good yields, while some have met with failures. Chicken manure harbours heavy population of pathogenic nematodes and harmful fungi including *Sepedonium maheshwarianum*, *Stachybotrys atra*, *Papulaspora* sp. and *Verticillium* sp. Growers should, therefore, avoid the use of this material under long method of composting.

b. Carbohydrate sources

These materials are essentially required to hasten the composting process, to balance the C/N ratio and also for the establishment of the bacterial flora in the compost. Molasses, wet brewer's grains, malt sprouts, potato wastes, apple and grape pomace can be employed as carbohydrate sources, since these materials provide readily available nutrients to microorganisms.

III. Nitrogen fertilizers

This category includes fertilizers like, urea, calcium ammonium nitrate, and ammonium sulfate. Nitrogen content of these fertilizers is very high (24-46%), which is released quickly, resulting in quick establishment of microflora.

IV. Concentrate meals

Animal feeds are generally kept in this category, which include wheat or rice bran, dried brewer's grain, soybean meal, cotton seed meal, castor meal, sunflower meal, etc. These materials supply both nitrogen and carbohydrates, which as in case of animal manures are released slowly. Nitrogen content may vary from 3-12% depending upon the source.

V. Supplements to rectify mineral deficiencies

In addition to carbon and nitrogen, *A. bisporus* also requires little quantities of potash, phosphorous, calcium and magnesium for its growth. Fertilizers viz., muriate of potash and superphosphate can be kept in this category. Besides this, gypsum and calcium carbonate can also be kept here. Gypsum also has stabilizing effect on ammonium content. An increased ammonium concentration is obtained with gypsum, which is an indicator of productive compost. Furthermore, gypsum serves as a calcium source for the mushroom and also for the oxalic acid produced by the mushroom mycelium, which gets converted into calcium oxalate. Requirement of phosphorous, potassium, and magnesium is generally met by chicken manure or horse manure when compost is produced by short or by indoor method. However, long method compost where chicken manure is not added addition of other materials may be required to meet the demand of these nutrients. For making compost for *A.bisporus* above materials should judiciously be selected keeping in view the nutritional requirement of *A.bisporus*, cost and availability of raw materials.

B. Formulations A large number of formulations are available with the growers and these are based on cost and availability of raw materials in the particular region. To initiate a composting process and to minimize the loss of dry matter during composting, 1.5-1.75 percent nitrogen is generally kept in the compounding mixture. The main objective of computing a formulation is to achieve a balance between carbon and nitrogen compounds. At stacking C:N ratio is adjusted to 25-30:1, which comes down to 16:1 after composting. N level in the compounding mixture at start should not be less than 1.5% as this will give improper compost with high C:N ratio and such compost will be easily attacked by cellulose loving fungi. It should also not be higher than 1.75% as such compost will be easily attacked by yellow moulds fungi and also more time will be required to finish the composting procedure. Known and estimated values of nitrogen and water contents of different materials viz., straw, chicken manure, wheat bran and other chemical fertilizers can be used as guidelines in computing formulations having desired balance of nitrogen and C: N ratio. Different batches of these materials can be tested for nitrogen for their correct estimates so that required quantity of these materials goes in a compounding mixture leading to productive compost. Formulations for white button mushroom compost should be so designed that composting mixture should have under mentioned percentage of different minerals on dry weight basis.

N	1.5-1.8%	CaO	1.5-3%
P ₂ O ₅	1.2-1.5%	MgO	0.4-0.5%
K ₂ O	2.0-2.3%		

Formulations having horse manure as one of the ingredients is termed as natural compost, while others are termed as synthetic composts. In addition to C and N, various other materials play an important role. Only in recent times importance of these minerals in mushroom cultivation has been realized. Chicken droppings have maximum amount of all the above elements and it should become an integral part of mushroom compost. An example as to how to arrive at standard formulation having desired N value is given in Table below.

Table. Nitrogen computation guidelines

Ingredients	Fresh wt (kg)	Moisture (%)	Dry wt (kg)	% N	N (kg)
Wheat straw	300.00	10	270.00	0.40	1.08
Wheat bran	15.00	10	13.50	2.00	0.27
Chicken manure	125.00	10	112.50	2.60	2.93
Urea	5.50	-	5.50	46.00	2.53
Gypsum	20.00	-	20.00	-	-
Total wt.	465.50		421.50		6.81

$$N\% = (6.81 \times 100)/421.50 = 1.61\%$$

C. Different Formulations Some of the formulations suggested by different institutions are:

DMR, Solan			
Wheat straw	300 kg	Wheat & paddy (1:1)	300 kg
Wheat bran	15 kg	CAN	9 kg
Chicken manure	125 kg	Urea	5 kg
BHC (10%)	125 g	Wheat bran	25 kg
Urea	5.5 kg	Gypsum	20 kg
Gypsum	20 kg		
Wheat straw	300 kg	Wheat straw	300 kg
Chicken manure	210 kg	Wheat bran	21 kg
Cotton Seed cake	21 kg	Cotton seed cake	12 kg
Gypsum	15 kg	Urea	7 kg
		Gypsum	15 kg
PAU, Ludhiana			
Wheat+Paddy straw (1:1)	300 kg	Wheat straw	300 kg
CAN	9 kg	Chicken manure	60 kg
Urea	3 kg	CAN	6 kg
Superphosphate	3 kg	Superphosphate	3 kg
Muriate of Potash	3 kg	Wheat bran	15 kg
Wheat bran	15 kg	Gypsum	30 kg
Gypsum	30 kg	Lindane	(5%)
BHC (5%)	250 g	BHC	250 g
Mushroom Research Laboratory, Solan			
(Long method)		(Short method)	
Wheat straw	1000 kg	Wheat straw	1000 kg
CAN	30 kg	Chicken manure	400 kg
Super phosphate	25 kg	Brewer's manure	72 kg
Urea	12 kg	Urea	14.5 kg
Sulphate of Potash	10 kg	Gypsum	30 kg
Wheat bran	100 kg		
Molasses	16.6 lt		
Gypsum	100 kg		

Many commercial units are making compost using only straw of wheat or paddy and chicken manure. For every ton of straw about 0.7-0.8 ton chicken manure and 0.05-0.15 ton gypsum is used. Gypsum is normally added at third turning but many units add it in the beginning or at second turning. Formulations recommended for long method can also be used under short or indoor methods. However, it is recommended that for short/indoor composting one should use chicken manure based formulations for economic gains. Further, one can go up to 70% addition of chicken manure/ton of straw depending upon its N-content. Other materials can be added to balance the N-level in the desired range.

C. Attributes of a good compost

A good compost should be dark brown in colour, should not be greasy or sticky, should have distinct sweet inoffensive smell, free from ammonia smell, should have 68- 72% moisture and pH 7.2- 7.8. There should not be visible growth of other undesirable organisms except for the fire fangs (*Actinomycetes*) and it should be free from insects and nematodes. As indicated earlier composting is essentially a fermentation process brought about by the activity of various organisms. Their activity and growth determines the quality of the compost produced since these organisms convert ammonical nitrogen to microbial proteins, which are ultimately utilized by *A.bisporus* mycelium for its nutrition. Beside above, quality and composition of base materials, aeration and moisture also determine the quality of compost. Various factors, which govern the quality of compost, are as follows:

a. Nitrogen content

Nitrogen content of the compounding mixture is very important. It should be 1.5 - 1.75% in the beginning (on dry matter basis). If the N content is kept below 1.5%, compost is not properly fermented and the temperature of the heap may not go beyond 55-60°C due to lesser microbial activity. The compost so produced will be yellowish in colour and light in texture and will not be selective to mushroom mycelium. Moulds like *Stachybotrys atra*, *S.alternans*, *Stilbum nanum* and *Doratomyces stemonites* may inhabit such compost resulting in poor yields. On the other hand if N content is kept above 1.75% level, C: N ratio will not be optimum and more of nitrogen will disappear from the pile in the form of ammonia resulting in the wastage of the nutrients. Such compost is invariably infested by *Sepedonium* spp. (yellow moulds), which may drastically reduce the yield. *Coprinus* spp. (Ink caps) and *Chaetomium olivaceum* (olive green mould) are also indicators of high nitrogen in the compost pile. N content of compost at the end of 28 days in long method compost is in the range of 1.75 to 2.0 %.

b. Carbohydrate content

During initial stage of composting free carbohydrates and nitrogen are utilized by the mesophilic flora and heat is generated in the process. Later on thermophilic flora takes over the mesophilic population. When the compost is cooled down, thermophilic flora can no longer grow due to low temperature while mesophilic flora also cannot grow since these organisms have already utilized most of the free carbohydrates. Normally there should not be any free or soluble carbohydrates present in the compost. Their presence is the indication of under composting and such composts are easily attacked by green mould (*T. viride*) or blackwisher mould (*D.stemonites*).

c. pH

This is an important parameter of *A.bisporus* compost. *A.bisporus* mycelium grows best at 7.2 - 7.8 otherwise growth of *A.bisporus* will be slow and white plaster mould (*Scopulariopsis fimicola*, *S.brevicaulis*) may invade such compost.

d. Moisture content

Optimum moisture content for the natural compost (i.e. compost made using horse manure) is about 65-67% while for synthetic compost it is 68-72%. If it is more than 72% at spawning there may not be proper aeration, as free space will be occupied by water. Under such circumstances anaerobic condition may

prevail resulting in killing of *A.bisporus* mycelium. Further, moulds like brown plaster (*P. byssina*), white plaster (*S. fimicola*) may appear in the compost.

e. Quality of raw materials

If raw materials especially wheat or paddy straw used in compost making are of poor quality (old and exposed to rains) it may result in improper compost. On such compost *Sepedonium* spp., *Alternaria alternata* and *Coprinus* spp. may appear resulting in low yield of mushroom.

D. Methods of composting

I) Long method of Composting - an old concept

Many seasonal growers are still preparing compost by Long Method of Compositing (LMC). Compost production by LMC is a very old concept and has been done away by advanced countries many decades back. It is presently in vogue only in few countries like India, China and Indonesia. LMC has following shortcomings.

Since compost is prepared over a period of 28-30 days dry matter loss of ingredients is more. We normally get 1.75 to 2.0 tons of final compost from one ton of dry straw. Compost is produced under outdoor conditions and hence invaded by many pests/competitors/diseases and hence not perfectly selective. Frequent sprays of insecticides and fungicides are required. Most of the ammonia is lost in the atmosphere resulting in low final N content of compost. Low yields. Not environment friendly

II) Short Method of Composting (SMC)

Long method of composting has many shortcomings as already mentioned. Growers in the United States around 1915 found that if compost prepared for *A.bisporus* is kept in shelves in growing rooms and subjected to high temperature (around 60°C) for sometimes gives higher and consistent yield. This process was later termed as “sweating out” and it laid down the foundation of pasteurization of compost. Based on the above principles/ findings, American Scientist Sinden and Hauser in the year 1950, 1953 proposed a new method of composting, where pasteurization became its integral part, which was termed as the short method of composting (SMC). This method of composting is being followed by most of the growers who are cultivating mushrooms round the year and has since revolutionized the mushroom industry. Short method of composting primarily consists of two phases: Phase-I: Outdoor composting for 10-12 days Phase-II: Pasteurization and conditioning of the compost inside an insulated room by free circulation of air under definite set of conditions. This phase lasts for around seven days

i. Purpose of pasteurization and conditioning

- a) It reduces the time of composting
- b) It converts ammonia into microbial protein, most of which otherwise goes waste in the atmosphere in LMC
- c) It conditions or sweetens the compost under definite set of temperature and aeration uniformly making compost more selective for the growth of *A.bisporus*
- d) It kills or inactivates insects/pests/diseases and competitors of *A.bisporus*, which if present hamper the growth of *A.bisporus* thereby reducing the yield
- e) Conditioning increases the biomass of thermophilic organisms especially that of *Scytalidium thermophilum*, which later on is utilized by the mushroom mycelium as food
- f) Through conditioning more compost per unit weight of ingredients is produced
- g) Conditioning and pasteurization increases the yield of mushrooms

During Phase-II steam pasteurization is done in a well insulated room constructed for the purpose. Boiler is required for the production of steam for proper maintenance of temperature inside the compost mass. Blower is required for the supply of fresh air and recirculation of ammonia and other gases for their conversion into microbial proteins. Details of pasteurization chamber are given in next section.

ii. Machinery required

Small farms would not require much mechanization owing to availability of cheap labour in the country. Also they have to handle little quantity of compost at a time, which otherwise can easily be handled manually. However, for a large export oriented unit (around 2000-3000 TPA), which handles the compost in bulk (around 30-40 tons of straw/day), mechanization of the operations viz., prewetting, turning, filling, emptying, spawning and bagging becomes necessary to hasten the process and to get a quality compost. Such farms also employ computers, which monitor and control the process of pasteurization and conditioning inside the tunnels. Following machines will be required for an export oriented unit.

a. Pre-wetting machine or pre-wet heap turner

This machine is used to blend loose or baled material with other compost ingredients such as chicken manure and horse manure as well as wetting of the mixed ingredients. The primary function of this machine is to turn and restack prewetted materials into long and wide heaps by tractor and front loaders.

b. Compost turner

The compost turner comes in varying capacities from 30-70 tons of compost handling per hour. It is fitted with a round stainless steel pick up drum, one spinner and one forming bore. The turner is generally mounted on 4 wheels, two of which are castoring wheels and rest two is powered, large diameter pneumatic wheels. Turner is usually fitted with a full width water spray pipe mounted at the front of the machine with water outlets over the full input width.

c. Pile forming case

This machine is used when the pile is formed for the first time. This is usually supported on four castoring wheels and is attached to the front of the compost turner which is pushed by the turner during pile formation.

d. Front end loaders

Bucket type loaders are employed for various composting operations viz, prewetting, and transportation of the compost during pile formation in combination of compost turner and forming case. They are generally attached with a tractor. Else, Bob Cat can be employed for the purpose.

e. Oscillating head filling machine

This is made up of two conveyer units mounted upon a self propelled chassis. The two conveyers are so designed that one feeds directly into the other from above. Conveyer, which is positioned above accepts the compost from the feed conveyers and transfer this compost to the conveyer positioned below. This is an oscillating type which fills the compost loosely in the tunnel over the entire width. The head filling machine comes in varying sizes suiting to the size of the tunnel.

f. Compost feed conveyers (2-3 units)

These are ordinary conveyer systems slightly elevated and can be coupled together to form a single conveyer system feeding one to the other during tunnel filling. The length and width of each conveyer is generally 7.5- 9 m and 0.6 m

g. Hopper regulator

This machine is required to feed the compost to the feed conveyers. It accepts the compost from the bucket of the front end loader and provides regulated output of the compost to the feed conveyer. This machine is used for filling the bags with spawned compost. The machine is equipped with a conveyer

with two filling stations. One or more of the above machines may be needed depending upon scale of operation, labour availability, type of raw materials used, etc Front-end loaders, hopper, conveyers and oscillating head filling machines are useful for any commercial unit.

Besides the above machines, small instruments like multi-probes digital thermometers, oxygen meters, ammonia measuring equipments and computers are also required for a mushroom farm to maintain quality and high productivity of mushrooms.

h. Bunker Filler

These days bunker fillers are available that can perform multiple functions and can be used for mixing ingredients, filling bunkers and even tunnels.

i. Tunnel emptying winch with combination of spawn dosing machine

This unit is employed for emptying the tunnel filled with pasteurized compost by means of a polyethylene glide and pulling nets. The winch is equipped with one net reel for pulling, the nets, two spinners and a chain conveyer for the discharge of the compost. Spawn discharging unit consists of twin spawn dispensers mounted over the full width of the compost flow on the discharge elevator.

j. Bag filling machine: For automated spawning and bag filling, this machine can be employed.

iii. Methodology: Compost by short method can be prepared by any formulation given in the text earlier. However, a formulation based on wheat straw and chicken manure is widely used in the country i.e. Wheat straw 1000 kg, chicken manure 500 kg, urea 15 kg, wheat bran 75 kg, gypsum 30 kg.

a. Phase-I or outdoor composting

This phase of SMC also starts with the wetting of the ingredients. Wheat straw and chicken manure are wetted thoroughly till they absorb sufficient water (around 75%). Leached water collected in a goody pit constructed for the purpose is regularly sprayed over the raw materials. After thorough wetting of the substrates an aerobic stack or a simple heap is made out of such materials. After 2 days the stack is broken, water is added to the dry portions and again a stack is made. Growers may provide artificial aeration to this heap and to the stack to be made later on for better results. They may pass up to 10 to 15 m³ of air/ton of wet compost/hour through the stack. This will result in achieving high temperature and more homogenous compost. To have artificial ventilation in the stack, working floor of the composting yard is provided with under stack aeration ducts connected with the required capacity small blowers installed at one end of the yard. These blowers blow small quantities of air regularly or at fixed intervals through G.I. or plastic pipes, which have small holes running length wise of the yard. Stack is made on these pipes. Pre-wetting and mixing of ingredients is a must before starting actual composting procedure on zero day and the stack made during this process are wide with low height of 3-4 feet.

On zero day, the stack is again broken and the entire quantities of other raw materials like urea and wheat bran are added, water is also added if required and a high aerobic stack is made. Dimensions of the stack are about 5' x 5'. Turnings can be done manually or by compost turners built for the purpose. Similarly the compost is again turned after every 2 days and gypsum is added at third turning. In all three to four turnings are given. On 8th – 10th day the compost is ready for pasteurization to be affected in tunnel. This marks the end of Phase -I.

Phase I can also be done in bunkers (as described in indoor composting) which have arrangement of under stack aeration through pipes or grated floor. After pre-wetting for two days and thorough mixing of all the ingredients, the material is shifted to bunkers where temperature of 75-80°C is achieved inside the compost. Air can be injected regularly or in pulses.

Characteristics of the compost after phase-I and before Phase-II

- Brownish throughout. Pieces of straw gleaming and wet
- Straw rather long but can be broken with some force

- Properly hydrated, around 72-75% moisture; if squeezed drops of water appears between the fingers
- Very heavy smell of ammonia. pH approximately around 8.2 - 8.5
- Still sticky and slimy, hands get dirty and wet
- Actinomycetes (fire fangs) not so apparent
- Nitrogen content between 1.5 - 2.0%; ammonia concentration around 800-1000 ppm

b. Phase-II

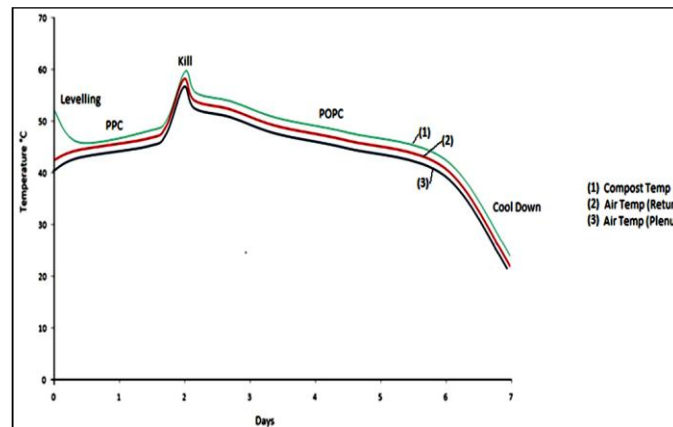
This phase of composting is generally performed in pasteurization tunnel in bulk. Phase-II process can be divided into two stages namely conditioning and pasteurization.

Conditioning

It can be divided into pre-pasteurization conditioning (PPC) and post pasteurization conditioning (POPC). During this phase of composting, whole of the compost mass is brought to a temperature range optimum for the growth of thermophilic flora (45-52°C). During this phase major part of NH₃ gets fixed in lignin-humus complex or as microbial biomass and excess of ammonia is released into the atmosphere. POPC again regenerates the lost thermophilic organisms during pasteurization. It has also been found that maximum ammonia generation takes place at 45-50°C, which corresponds well with optimum temperature range of majority of thermophilic flora. Compost should not be conditioned below 40°C, as some mesophilic fungi may set in at this temperature rendering compost unsuitable for mushroom growth specially *A.bitorquis*. Besides keeping compost at a particular range of temperatures (45-52°C), during this phase enough of oxygen is supplied (O₂ concentration above 10%) to the compost mass to maintain fully aerobic conditions. Both pasteurization and conditioning make the compost most selective for the growth of white button mushroom at the expense of other harmful competing organisms.

Pasteurization

Main purpose of pasteurization is to kill or inactivate harmful organisms. They are eliminated when the compost is subjected to a temperature above 55°C for certain period when humidity in the compost and surroundings is high. Therefore, use of live steam to heat up the room and compost sometimes becomes essential. It has been found that compost is pasteurized properly if it is kept at 59°C for 4-6 hours. Temperature above 60°C is harmful as this temperature may kill all kinds of organisms including thermophilic fungi very essential for governing the phase-II of composting. Activity status of the compost is also very important to achieve pasteurization temperature. If it is active compost, its temperature starts rising immediately after filling and may rise by 1°C per hour and the required temperature of pasteurization can be achieved in few hours only by self-generation of heat. Pasteurization of the compost can either be done soon after room/tunnel filling or after few days.

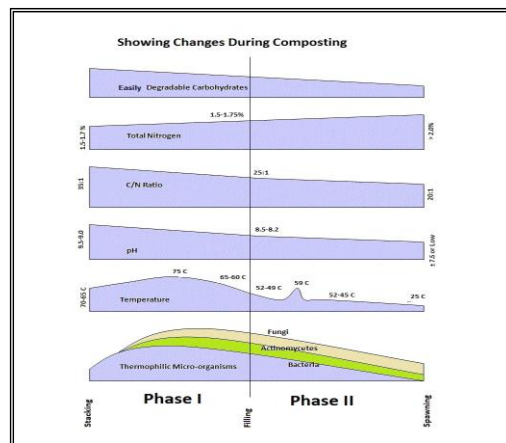


Process

The compost is treated in bulk inside a specially built chamber known as the tunnel. The compost is filled in the bulk chamber up to the height of 2- 2.2 meters in such a way that one square meter of space occupies approximately 900-1000 kg of compost. Several temperature sensors are placed at different points of the tunnel to measure the temperature. One sensor is placed below the plenum in the ventilation duct below the grated floor, one to three are placed inside the compost mass and one or two above the compost for air temperature. Immediately after filling, all the doors are closed and the blower is switched on to bring the air in plenum, compost and air above the compost at a uniform temperature (around 45-48°C). There will be a little difference in temperature at all the three places and this difference may be 1-3°C. Levelling off may take 4-5 hours and at this stage no fresh air is generally introduced in the tunnel and air introduced through the leakage of the dampers and ducting would suffice the purpose. After levelling that is to say after 4-5 hours (more in case of bigger tunnels >15 tons) of filling the tunnel, we will start Pre Pasteurization Conditioning (PPC). This is to increase the population of thermophilic fungi at this stage, which will demand more oxygen for their growth and multiplication and this may reach above 15% of the total gaseous volume inside the tunnel. Fresh air is therefore introduced in the tunnel through the dampers (10% opening). Now the compost is kept between 45-52°C for two days. Two days after conditioning, the compost is now ready for pasteurization. Now opening of the damper is narrowed down, which will gradually increase the temperature of the compost by approximately 1°C/h. Required temperature (58-59°C) of compost needed for pasteurization may reach in 10-12 h by self-generation of heat. The difference in the temperature above the compost (air temperature), inside the compost and plenum (below the compost) should be as less as possible and may not exceed 3°C. Some quantity of steam can also be used if temperature is not rising. This process is called pasteurization or killing. Duration of the pasteurization is normally 4-6 hours. It will eliminate harmful insects, nematodes and competitor moulds from the compost and at the same time will preserve the nutrients in the compost, which can effectively be utilized by *A. bisporus* mycelium. Temperature can be monitored or regulated through automatic computerized systems available in the international market. A low cost alternative has been developed at the Directorate wherein one can set the minimum and maximum temperature and as soon as the temperature goes above or below the set temperature range, there is siren and corrections in tunnel parameters thereafter can be made manually. After killing, fresh air is again introduced / increased in the tunnel and temperature of the compost is brought down @ 1.5°C/hour and finally maintained between 45-48°C till there is no detectable smell of ammonia (less than 10 ppm) in the compost. This phase is known as post pasteurization conditioning (POPC) of the compost, which is normally accomplished in 3-4 days. Temperature of the compost is gradually brought down to 25- 30°C after conditioning by introduction of fresh air in the tunnel and when this temperature reaches, the compost is ready for spawning.

Above method of pasteurization is recommended for the commercial tunnels having more than 15 tons output of the compost. Smaller tunnels while adopting above procedure may require frequent injection of steam during PPC and especially when pasteurization is affected, this increases the cost of production of compost. Such tunnels may resort to traditional pasteurization wherein leveling is done at higher temperature (around 50°C) and after that opening of the damper is so adjusted that compost temperature starts rising and it attains pasteurization temperature mentioned as above. Usual conditioning is done afterwards for 4-5 days or till the period when compost is free from ammonia and spawning done as usual. At the end of conditioning (at spawning) compost should be dark brown in colour with a full coating of white powdery mass due to abundant growth of actinomycetes. This is a sign that Phase-II was performed in a perfect manner with abundant supply of fresh air. Phase-II process is almost a biological oxidation (90%) and hence here O₂ and temperature play very important role. It is advisable to connect temperature probes with a computer or data logger for round the clock changes/monitoring of the temperature. Further, gadgets are available in the market to monitor ammonia concentration and oxygen level inside the tunnels. These can also be installed in the tunnel to monitor above gases round the clock. The fresh air inlets are fitted with 2 micron washable HDPE filters. As the composting proceeds there is loss in biomass. In phase-I there is about 30% loss in weight and in phase -II, 20-25 % loss in weight

takes place. As a result from the standard formula of one ton wheat straw and 0.5 ton chicken manure, we can get about 2.5 tons of final compost.



Characteristics of the compost after Phase-II

- Dark brown in colour, full of thermophilic fungi and actinomycetes. It is soft, straw breaks rather easily.
- Moisture around 64-66%. No liquid oozes when squeezed firmly
- Pleasant sweet smell
- No stickiness. Hands stay clean and dry
- N content > 2%
- Ammonia below 10 ppm

Advantages of bulk pasteurization

- More compost per unit size of the room can be treated at a time.
- The cost of pasteurization in tunnel is less.
- Same tunnel can be utilized for spawn run in bulk, which gives effective use of the space.
- Yield per unit weight of compost is generally higher.

III. Indoor Composting

1. Facilities required

a. Composting yard

In indoor composting Phase-I is performed indoors and hence requirement of composting yard is greatly reduced. A small composting platform is required for pre-wetting and mixing of the ingredients, which is mainly performed either by front-end loaders or by pre-heap turners by big commercial units. A platform of the size 60x60x14 ft (h) would suffice the purpose for a medium size farm (250 TPA).

b. Phase-I bunkers

These are specially built non-insulated tunnels having full width opening at the front. Dimension of the bunkers would depend upon the output of the compost required. Generally the bunkers are 1.25 to 1.5 times more the size of the phase-II tunnels. It has a plenum (ventilation duct). A perforated concrete floor is constructed above the plenum, which is serviced by a centrifugal fan having $\frac{1}{4}$ the capacity of phase -II blower, which means that a ventilator having air displacement of 50 m³/hour/ton of compost at 50mm WG water pressure would suffice the purpose. Alternatively, the bunkers have no plenum and several pipes are buried in the floor along the full length of the bunkers having small holes (5-8 mm dia). These pipes are converged into a manifold, which in turn is connected to a high-speed blower fan (around 1000

pascals). A timer is usually attached to the blower, which pulsates the air in the bunker periodically as per the setting of the timer. A minimum of 2 such phase-I tunnels (bunkers) are required.



c. Phase-II tunnels

Structure and design of these tunnels are the same as required in case of short method of composting.

2. Selection and mixing of ingredients

Selection of the raw materials for indoor composting is very critical and should have the following qualities: (i) High bulk density, (ii) Good structure and texture, (iii) Perfectly mixed raw materials, (iv) Well balanced chemical composition and (v) High level of nutrients

3. Procedural requirements

Two methods, INRA method (double phase high temperature process) and Anglo Dutch method (single phase, low temperature process) are prevalent in most parts of the world giving almost equal yields. This Directorate has developed a method combining the two methods as mentioned above. Methodology developed is presented below: For preparing compost by this improved method of composting, ingredients say – wheat straw 1000 kg, poultry manure 500 kg, wheat bran 70 kg, cotton seed cake 20 kg, and gypsum 40 kg are first thoroughly mixed in dry form. They are then thoroughly wetted so as to achieve around 75% moisture percentage. Runoff water should regularly be collected and sprinkled over the wetted straw. On the following day these wetted ingredients are then spread over the composting yard (around 8-10" height) and trampled hard by running Bobcat several times over the wetted ingredients or by other means so as to increase the bulk density of the ingredients and also to shred the straw. Wetted straw together with other ingredients is then made up into heap and left as such for 48 hours. Temperature in the heap may rise up to 55-60°C. On the following day, material is again flipped to bring the uniformity and proper mixing and transferred to phase-I bunker, for phase-I operation. This material will weigh around 4 tons and height of the compost in the bunker is kept up to 1.8-2 meters. Temperature sensors are installed on the top and in the Centre of the pile in the bunker and blower fan switched on @ 5 min/h with the help of a timer installed for the purpose. Temperature will rise to 60-65°C after 24 hours in the centre and 48-52°C at the bottom, sides and on top of the compost. After 24 hours air flow inside the tunnel is increased to 10 min/hour. This will further increase the temperature in the centre of the compost between 72-75°C while it will remain same in other parts of the compost mentioned as above. No foul smell will be noticed while performing phase-I operation in the bunker, however little bit ammonia smell will be there. After 3 days of partial fermentation in phase-I tunnel, entire compost mass is taken out and a complementary turning is given, more water can be added if required and is transferred to another bunker or to the same bunker at the same sets of conditions mentioned as above for 3-4 days.

Total period of phase-I operation in the bunker should normally last for 6-8 days. Afterwards compost is transferred to phase-II tunnel for usual phase-II operations to be completed in 6-7 days.

a. Composting schedule

-4 day: Mixing and wetting and of the ingredients out doors

-3 day: Turning, trampling by Bobcat and thorough mixing of the ingredients, addition of water.

-2 day: High aerobic heap

0 day: Filling in the phase-I bunker

+ 3 day: Emptying the bunker, turning and mixing of the compounding mixture and re-filling the compost in another phase-I bunker

+6 day: Phase-I operation over and compost transferred to phase-II tunnel

+ 12 day: Phase-II operation over

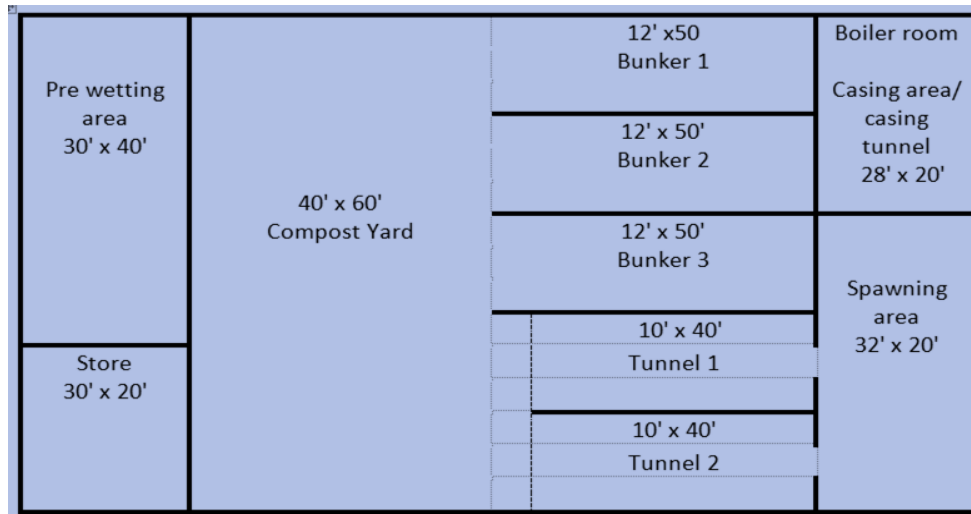
As temperature inside bunkers sometimes rises above 75°C, it may be desirable to add some inoculum in the form of readymade compost to the ingredients at the time of filling the tunnels for phase-II.

b. Advantages of using bunkers

- Requirement of composting yard is reduced
- No emission of foul smell
- Number of labourers and cost of production reduced
- Duration of composting reduced
- Reduced effects of weather and seasonal variations
- More compost per unit weight of the ingredients
- Higher yields
- Compost turner is not required

Farm Design

White button mushroom is a temperate mushroom requiring cooler climate for its growth. It is an indoor crop and is an ideal tool in converting agricultural wastes into proteinaceous food. In early days its cultivation was mainly confined to the hills. In the eighties growers realized the potential of this crop and started its cultivation in the northern plains in the winter when the climate was suitable for its growth. Many entrepreneurs in the plains further ventured and started its cultivation round the year by employing artificial cooling facilities (chilling stations). Today its cultivation is done throughout the country. Some are doing it seasonally while many of them have preferred to go for round the year cultivation. Today India boasts of having world's biggest farm, the Agro Dutch Foods Ltd, Lalru Punjab and many more environment controlled units exist in different parts of the country cultivating this mushroom round the year. Mushroom being an indoor crop does not require arable land, except for some non-agricultural land to build the infrastructure for preparation of substrate, raising of crop, preparation of spawn and postharvest handling. As mentioned above this mushroom is grown seasonally and in environment controlled cropping houses and both require building of basic infrastructure. Seasonal growing is done for 3-4 months when outside temperatures are favourable for the crop, i.e., during winter months in N.W. plains and from September to April in the hills. Seasonal cultivators of this mushroom are using traditional methods of its cultivation and are mainly cultivating this mushroom in the thatched structures employing long method of composting. They usually take single crop in the entire season and are harvesting 12-15 kg mushrooms/ 100 kg compost.



Environment controlled units are cultivating this mushroom round the year by having suitable infrastructure at their disposal which includes a modern composting yard having bulk pasteurization facilities. Of late few of them have shifted to indoor composting while new upcoming units have chosen to produce their compost entirely by indoor method. Besides these facilities they are having insulated cropping rooms and other ancillary structures required for mushroom cultivation. Few of the bigger units are having their own spawn lab and processing unit as well. An entrepreneur can start mushroom cultivation modestly using seasonal growing houses and after gaining sufficient experience can shift to round the year cultivation employing suitable climate control facilities. Suitable infrastructure including different machineries are required at the farm to carry out different operations to govern the whole process of cultivation in such a fashion so that one gets optimum returns from his farm in this competitive environment. The one who designs the farm in most scientific manner looking to the need of the crop and easy accessibility to the different infrastructure for their operation convenience in less space, utilizing less money will gain handsome returns in the years to come. Present chapter would deal in detail the infrastructure and machineries required for the seasonal and environment controlled units.

A. Selection of Site and Pre-Requisites Before selection of site, the following points have to be taken into consideration for greater operational efficiency and cost effective production of mushrooms at the farm: 1. Chosen site should preferably be away from the municipal limits and entrepreneur should purchase sufficient land in one go looking to the future expansion. 2. The site should be serviced by a motorable road, or nearer to a road head to reduce costs on transportation of raw materials to the farm/finished product to the market. 3. Plentiful availability of water at the site either through a perennial source or should have sufficient underground water. 4. Easy availability of raw materials especially straw and poultry manure around the chosen site at cheaper rates in the area. 5. Availability of cheap labour in abundance. 6. Uninterrupted proper power supply at the chosen site. 7. Nearness to the market for the proper disposal of the produce.

B. Components of a Mushroom Farm For round the year cultivation of this mushroom employing environment-controlled condition a medium size plant would require under mentioned components.

Compost production unit will have under mentioned main components:

- a Pre wetting area:** For dumping of raw materials and their pre wetting (uncovered).
- b Composting yard:** For making piles out of the wetted materials (covered)
- c Phase-I bunker:** For phase -I composting (in case indoor composting is employed).
- d Phase -II tunnels:** For performing pasteurization and conditioning of the compost.
- e Casing soil chambers:** For pasteurization of the casing soil.

f Spawning area: For spawning of the prepared compost Besides above certain ancillary rooms like boiler room, underground service room, store room, workers room, etc. would also be required. Machineries viz., boiler, blowers, air handling units, gratings, digital thermometers, compost retaining boards, ventilation system for phase -I bunkers would be required by a medium size farm (up to 200 TPA). Large farm besides above may require a front end loader (Bobcat) and other compost handling equipments including turner, filling line, etc.

5. General layout/location of various units: The layout is so planned that all the infrastructures required to be built are accommodated in least possible land without over looking mushroom cultivation requirements. The general layout of a mushroom farm has to be carefully planned after selection of the site, keeping in view the several factors including accessibility of road to the composting yard as raw materials are to be dumped here for their processing to the compost. Wind direction is also kept in mind for choosing the location of the composting facilities. During most of the time of the year wind should flow from cropping area to the compost yard and not vice versa. Phase-I bunkers are constructed in line nearer to the phase -II tunnels for their operational convenience and also to avoid heat losses. The bulk chambers are built nearer to the phase-I bunkers. Both these structures are preferably built away from the road at the distant end of the yard so that the distant end of the phase -II tunnels opens nearer to cropping rooms and away from composting yard. The cropping rooms are built away from composting area for reasons of cleanliness and avoiding contamination by pests and pathogens. The casing pasteurization chamber is built nearer to the composting yard or within the composting yard with small platform for preparing the casing soil. Enough space for future expansion of composting yard, construction of more phase-I & II chambers and growing rooms should be left vacant for planned development of a mushroom farm in a phased manner. Spawn unit is built far away from the composting yard and nearer to the cropping area. Processing unit can be a separate entity or can also be built within the building housing cropping rooms. The foundation of the buildings is dug on the firm ground. The underground water pipes, electrical cables and sewers are laid well before the actual construction starts. The entire site area should preferably be fenced or brick walled for security reasons. In areas where land is scarce, double story cropping houses can be built to economize on space. The cropping rooms are generally built in double rows with a path/gully in between for various operations and services.

a. Composting unit: The components of composting unit will depend upon the method of compost production chosen. If one is going for indoor compost production, in such a case requirement of composting yard will be greatly reduced and it will be 1/3 of the normal yard required when one has chosen SMC. Now a day's trend is for indoor compost production due to environment legislation. In such a case a small pre wetting area, and small covered composting yard would be required with minimum of two-phase-I bunkers and one phase-II tunnel. Size of all these structures would depend upon the production targets of the unit and size and numbers of the tunnels.

b. Pre-wetting area (PWA) (lagoon) This area is constructed nearer to the road. It is a simple cemented structure having a saucer like depression in the center so that it looks like a lagoon and water remain collected during the pre-wetting of the compost ingredients. Center of the lagoon should be around 1 ft deep. Excess water of the lagoon is collected in a goody pit built specially for the purpose at a convenient place around PWA for its reuse. Floor of the PWA should be such that it can withstand the load of the front-end loader while performing the wetting operations. It is usually not covered and is open to the sky. PWA terminates in the composting yard. Water connection with 2"-3" dia. pipe should be available in PWA permanently with additional portable hosepipe for use during pre-wetting. One dewatering pump with a hose should be installed in the goody pit to pump out the run-off water for its reuse during pre-wetting. Water in the goody pit may be aerated continuously to avoid foul smell.

c. Composting yard composting yard has to be done as it has to withstand the load of heavy machines. The floor is given a run-off of 1 cm per running meter away from the bulk chamber and towards the goody pit end. The roof of the outdoor composting platform is built on tresses or RCC pillars 16 ft high

with a GI or any other suitable roofing. The covered composting yard should be big enough to hold maximum compost stacks for phase-I of composting. When adopting indoor compost production wetted ingredients are just made up into a heap for 3-4 days and do not require rick formation in such a case a small platform can suffice the purpose. However, a large composting yard would be required if SMC is adopted. The composting yard is required for phase-I of composting. It is a prerequisite when one is going in for short method. The composting yard should necessarily be a covered shed with 2-3 ft sidewalls on the two sides (length wise) where rain will not interfere in the normal process of composting. The foundation of the composting yard should be laid on a firm ground and it should necessarily be reinforced if mechanization is to be done.

One ton compost occupies about one meter length of the composting yard, with an extra space of 2-3 m left on each side for turning with machines. Two bulk chambers will have a platform with 10-15 m width. For two bulk chambers of 25 tons capacity each, a composting yard of 25 m x 13 m should be good enough to concurrently run phase-I operation at a time for both the chambers. A drain should run on the two sides of the platform to facilitate periodic cleaning. A few three phase 15 amp. power connection should also be provided at the composting yard for operating machines like hopper regulator, compost turner, filling lines, etc. The yard should be well lighted with tube lights and strong searchlights to facilitate round the clock operations at the composting yard. An overhead water tank is necessary, particularly where water is scarce, to store water for timely operations. The floor of the composting yard for long method of composting should be simply cemented/brick layered with a low cost roofing of high-density polythene fixed on iron tubular structure or it can also have thatched roof. In practice (90%) of the farms cultivating this mushroom seasonally are preparing their compost in the open fields and do not have any specially built composting yard built for the purpose. However, such growers are facing lot of disease and pest problems. We recommend that the compost by long method by seasonal growers should at least be prepared on a cemented platform- let it be open to the sky.

d. Phase-I tunnels (bunkers) This facility is required when indoor composting is employed at the farm. These are specially built non-insulated tunnels having full width opening at the front. Dimension of the bunkers would depend upon the output of the compost required. Generally the bunkers are 1.5 times more the size of the phase -II tunnels. It has a plenum (ventilation duct) constructed below the actual floor. A perforated concrete floor having around 1 cm openings at a distance of 1ft each to the entire floor area is constructed above the plenum or it has simple RCC /steel gratings having 20% opening to the entire surface area of the tunnel which is serviced by a centrifugal fan having 1/4th the capacity of phase two blower which means that a ventilator having air displacement of 50 m³/hour ton of compost at 50 mm WG water pressure would suffice the purpose. A plenum floor involves pressurizing the entire airspace beneath the concrete floor, allowing the air to move up into the substrate through the holes or through series of slates. Alternatively the bunkers have no plenum and several pipes (5-15 cm dia) are buried in the floor along the full length of the bunkers having small holes (5-10 mm dia) at a distance of 15 to 30 cm each. These pipes converge into a manifold, which in turn is connected to a high-speed blower fan (around 1000 Pascal). A timer is usually attached to the blower, which pulsates the air in the bunker periodically as per the setting of the timer. A minimum of 2 such phase-I tunnels (bunkers) are required.

A bunker for 20-25 ton compost output at the time of spawning may have the dimensions 45 x 10 x 8 with 9 pipes of 2.5 m dia. at distance of 1 ft. (6" from the wall). To equalize the pressure either the pore size may be increased or distance between the holes may be gradually decreased from 1.5 ft. to 9". These 9 pipes are linked to a bigger pipe of about 6" dia. which in turn is linked to a centrifugal blower.

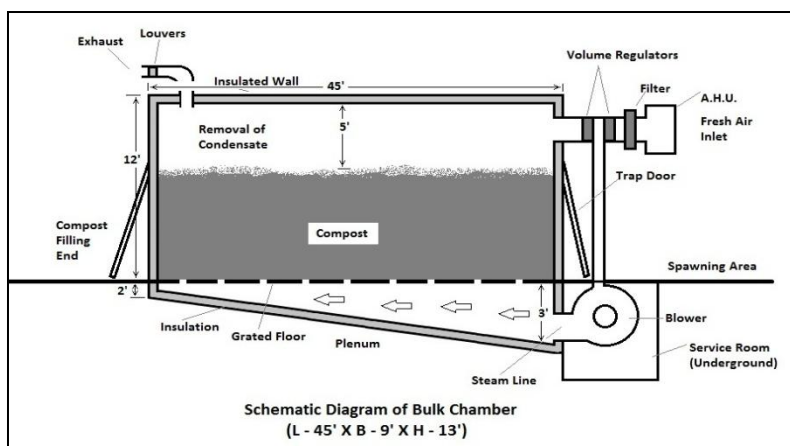
i. Phase II pasteurization tunnels: A modern farm employing either indoor method or SMC essentially requires this facility. The bulk pasteurization chamber is principally used for phase-II of composting for pasteurization and conditioning of the compost. For this purpose, an insulated chamber is built with facility for steam injection and controlled recirculation and fresh air entry in the tunnel through a blower. The insulated chamber is built with purpose of cutting off the external environment and simulating a

desired environment inside for controlled fermentation of the compost ingredients. In Bulk pasteurization chamber compost is handled in bulk inside the tunnel or chamber and hence the name bulk chamber.

The compost after phase-I is filled into specially built chamber, which is properly insulated and provided with steam connection and air blowing system for re-circulation. The compost is filled in the chamber on top of its grated floor built over the plenum. The plenum has an air circulation duct used during pasteurization/conditioning. The bulk chamber should be constructed on one end, (away from road) of the composting platform. One end of the bulk chamber should open into the platform and the distant end in the clean spawning area. The foundation of the bulk chamber should be dug on a firm base ground. The floor must be laid with a good run-off provided with a drain to facilitate cleaning. It is pertinent here to note that this floor is given a slope towards the service area end (blower end). A large tunnel will be around 90 cm deep towards the blower end while it will be around 15 cm deep towards other end (filling end). Floor should be properly insulated with thermocol/glass-wool 5 cm thick (15 kg/m² density). The insulation is covered with isolating membrane of PVC sheeting followed by 5 cm cement floor and finally the finish. Such floor is constructed for both cropping room and the chamber. The walls should be 9" thick (one brick lengthwise) built over the concrete foundation.

The length and breadth of the bulk chamber will vary, depending upon the amount of compost to be treated in the chamber, with the height of 13 ft, the roof is made of 4" thick RCC. The walls, ceiling and the floor below the plenum are insulated with 5 cm thick insulating material (15 kg/m² density) necessary for effective insulating effect during pasteurization and conditioning of the compost. Required K value of the insulating material should be around 0.5-0.6 kcal/ m²/h. Air leakage in bulk chamber must be prevented at any cost. The bulk chamber has two floors one is real insulated floor while another false or grated floor, which is laid above the actual floor or plenum over the ventilation duct. The grated floor must allow the air to pass through, for which approximately 25-30% of the floor area is left in the form of gaps for ventilation/circulation of air and steam. The plenum is divided with a perforated brick wall (one or two) in the centre for supporting the grated floor. The gratings can be made of wood (painted with bituminous paint), coated iron strips mounted on angle iron frame or with concrete beams. Alternatively a concrete floor can be poured over the plenum as in case of phase -I tunnels having openings. If nylon nets are to be used for mechanical filling and emptying, then cemented grated floor with appropriate RCC strength is built specially for the purpose. The doors of the bulk chamber are made of angle iron or wooden frame with 2-3" insulation in the middle and covered on both sides with aluminum sheets, else they can also be made up of puff panels. The chamber will have two exhaust vents, one for recirculation exit and the other for exhaust of gases on introduction of fresh air via dampers. The steam line is also connected at the entry point of the blower. The walls and ceiling can be damp proofed by coating bituminous paint on inside over the cemented surface, which will also serve as an effective vapour barrier. The grated floor inside and the work floor outside should be of the same height for operational convenience especially when tunnel has to be filled mechanically.

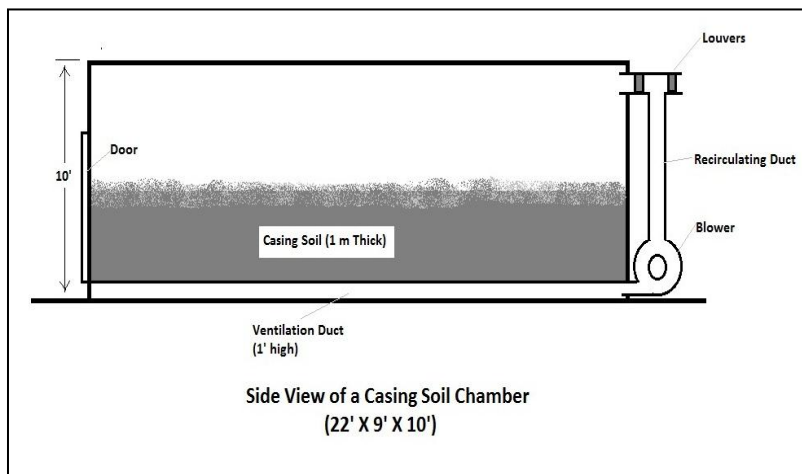
Two types of tunnels (bulk chambers) are in use, two door bulk chambers and single door bulk chambers. In the single door bulk chamber, the same door is used for filling and emptying and the other end is utilized for fixing installations (blower, etc.). In double door bulk chamber, one door is used for filling (which opens into the composting yard) and the other for emptying (opening into the sterile spawning area). The bulk chamber can be filled/emptied manually or by conveyer belts. The uses of machines for filling/emptying are labour saving, time saving and ensure homogenous filling as well as maintenance of absolute cleanliness during operations. For mechanical emptying two nylon nets are used, one fixed over the RCC grated floor (gliding net) and the other moving over the lower net (pulling net). The compost when brought out is fed into the spawn-dosing machine where requisite amount of spawn is mixed with the compost and the seeded compost is then poured into clean polythene bags for transport to the growing rooms. The dimension of the tunnel for producing 20-25 ton of compost is 36' x 9' x 13'. One may replace the plenum with plastic pipes fitted with spigots. The centrifugal fan can be placed at the bottom as well as on the roof depending upon the space and design.



e. Air handling units of the tunnel (AHU) For effective pasteurization and conditioning of the compost in the tunnel specific requirements of air and ventilation are to be met, which are generally met by providing/ installing AHU in the tunnels. Effective pasteurization and conditioning is attained when 150-200 m³ air per ton of compost per hour is blown through the compost mass. For this purpose high speed centrifugal fan is chosen and is placed on the slope end of the ventilation duct in the underground service area. Compost is spread over the plenum on the grated floor in about 2-2.2 meter thick layers. Nylon nets are generally placed under the compost if mechanization is necessary. These together give a resistance of around 60-65 mm WG during pasteurization taking together the resistance of the air ducts, the in and out openings, the perforated floor, etc. the static pressure of the blower fan should be around 100 mm WG at 150-200 m³ air per ton of compost per hour. Blower fan must be well protected internally and should be made up of sheet steel. Aluminum is ideal for air ducts and should at least be 2 mm thick and there should not be any leakage in the duct system. Ducts are generally insulated with glass wool or any other suitable material. Fresh air is regularly required in the tunnels and since this air is drawn from the open atmosphere, chances of fungal spore's contamination are likely and hence the incoming air in the ventilation duct should be filtered and should pass through 2 mm fungal spore filters. The pre filters and filters should be washed at regular intervals. The inlet and exhaust openings must be fitted with a flap valve, which opens only when positive pressure is created inside the tunnel. The dimensions of inlet and exhaust openings should be the same. Since, India is a tropical country where temperature during summer months goes above 45°C. Cooling of compost for spawning during this period becomes difficult by simple introduction of fresh air. Special cooling arrangements are therefore required to be made in the AHU of the tunnel for this purpose. A ten ton capacity cooling equipment or cooling coils from the central chilling plant is installed at the top of the AHU or such coils can be fitted in the blower section of the AHU. This arrangement is very effective in cooling of the compost in tropical areas during summer months. Installation of such facilities however requires heavy investment. Compost during these months can satisfactorily be cooled during nights when the temperature is low.

f. Casing pasteurization chamber Casing pasteurization chamber is just a mini bulk chamber. It has all the necessary components as required for the tunnel. Only difference is that the plenum is not having any slope and capacity of the blower for proper steam injection and its uniform distribution inside the casing mass is around 1/4 the capacity of the tunnel. The size of the chamber will depend upon the size of the compost chamber and the size of the growing rooms. One chamber load should provide casing for one compost lot from each tunnel. The casing inside the chamber can be treated in the bulk and in such case it is filled up to the height of 90 cm only as against the tunnel where compost is filled up to the height of 2-2.2 meters. Else casing after wetting is filled into the perforated wooden/aluminum trays which are stacked one over the other inside the chamber and steam treated at 65°C for 6- 8 hours. This chamber can

be built near to the composting yard or within the composting yard with a separate casing mixing platform.



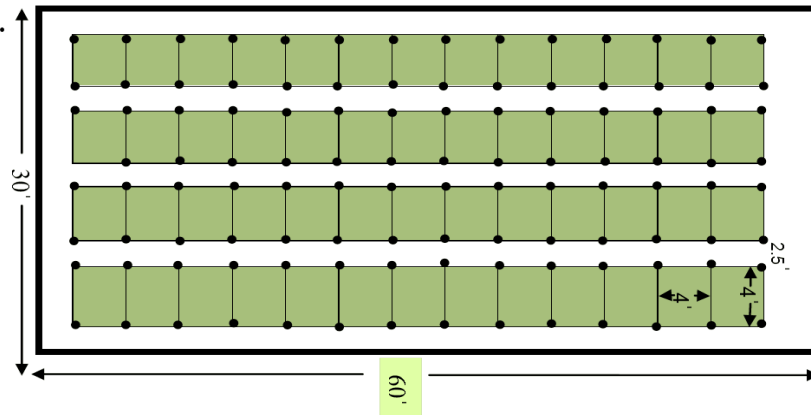
3. CROP PRODUCTION

Design of cropping Rooms

Since mushrooms are grown indoors under simulated environment specially created for mushroom growth, the cropping rooms are required to be built specially for the purpose. Two types of cropping rooms are built suiting to particular requirement - those required for seasonal growing and those for environment controlled growing round the year.

Seasonal cropping rooms

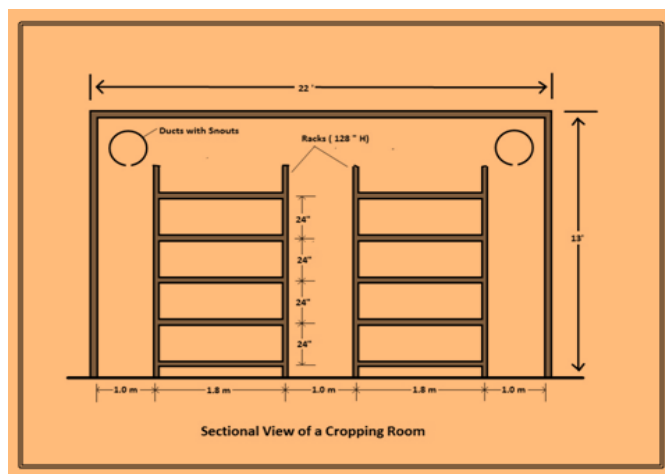
Seasonal cropping rooms are simple rooms with modifications for maintaining various growth parameters. These cropping rooms will have a cemented floor, cemented walls, cemented ceiling or a false ceiling with arrangement for forced air circulation inside. The seasonal cropping rooms are built of simple brick walls with roof made of asbestos sheets and a false ceiling. The room is more or less made air tight to make the air handling system work effectively for obtaining necessary air changes during growing. No insulation is required for seasonal growing rooms, as it will not allow heat dissipation from the room efficiently. These simple rooms are used for seasonal mushroom growing, coinciding various phases of growth with prevailing outside temperatures. No energy is generally used for heating/cooling of the rooms under seasonal growing conditions. However few units in plains have installed heavy-duty coolers to bring down the temperature in summer conditions. The cropping rooms for seasonal growing can also be made with a thatched roof and a false polythene ceiling. The door is installed on one end and the exhaust vents on the opposite end of the door. The mushrooms are grown on beds made out of bamboo sticks and sarkanda stems (a plant abundantly growing as a weed in North western plains of India). These growing rooms can also be built as low cost structure, steel pipe frame with high density polythene covering from outside. The real low cost growing houses built in rural areas are made of walls, roof and door of sarkanda. The mushroom houses made with bamboo frame and paddy straw have given good results conditions for seasonal growing. Design of one such hut is given below



Lay out of mushroom shed commonly used for seasonal cultivation

Environment controlled cropping rooms

The environment controlled cropping rooms are built like hermetically sealed chambers where the air movement is controlled either manually or semi automatically with mechanical control systems. These cropping rooms are appropriately insulated and the dimensions of a cropping room are determined by the amount of compost to be filled into the room. Rooms with greater length and narrower width gives better results as far as air handling inside the room is concerned. A cropping room, with a capacity to take compost from one bulk chamber, is considered advantageous as one bulk chamber load can straightaway be filled into one cropping room. Further, cropping cycles to be taken will determine the numbers of growing rooms in the unit. Now a day's 60 days cropping cycle is generally taken and in this manner a minimum of six crops are taken / room in a year. In such conditions a minimum of 12 rooms are required to have constant supply of mushrooms from the unit round the year. In this case every room is filled with the spawned compost after every 5 days. Both tunnel and cropping rooms of 20- 25 tons compost capacity are considered to be operationally efficient, as the filling/ emptying operation and spawning can conveniently be done in one day when machines are not to be used. However, bigger units may have the growing rooms handling compost to the tune of 60 tons or more. Growing rooms are such designed that maximum compost can be accommodated in least possible area without over looking to the mushroom growing requirements. To give an example a room size of 57 x 19.5x 12.5 ft can easily accommodate 20- 25 tons of compost when cultivation is done in shelves or bags (Two rows of stands with 5 tiers, each 1.5 wide; and 3 paths each one metre = 6 m or 19.7 ft). The foundation of growing rooms should be laid on dry and firm ground. The floor is laid as per normal standards. The walls will be made of one brick thickness (9" thickness) and ceiling made of 4" thick RCC. The growing rooms will have a single insulated door and 2 vents for exhaust on the back wall 2-3 ft above ground level. One opening is provided on top of the door for entry of the Air Handling Unit (AHU) delivery duct or for fresh air intake inside the room through AHU. The walls, ceiling and floor should be insulated with 5 cm thick insulating material (Thermocol). The room should be made airtight and all leaks closed to prevent ingress of heat, flies, etc from outside. The cooling, heating and forced air circulation in the growing room is done via AHU installed for each cropping room individually or for whole of unit. The floor and walls of the cropping rooms should have a smooth finish.



j. Structural details special to cropping rooms

i. Floor

The floor must be well laid out and should be strong enough to take the heavy load of metal racks to be kept inside for growing mushrooms. The floor should be insulated with insulating material 5 cm thick (sheets of thermocol or glass wool or polyurethane). The insulation should be protected by a PVC sheeting, below and above, against moisture. It is then covered with wire mesh and finally 5 cm thick concrete floor is laid on top, which is given a smooth finish. The floor should have slight slope towards the entry point for discharge of cleaning water and placement of formalin trough for foot wash. The trough is connected near the wall to an exhaust drain to carry washings from the room. The water discharge hole is protected at this point to prevent leakage of air from the growing room. PUF pads can also be used specially in place of wall between rooms.

ii. Walls

The walls are made of brick 22.5 cm thick, which are given a smooth finish with cemented plaster. The insulation sheets are fixed on the walls (5 cm thick thermocol, glass wool/polyurethane), with the use of hot coal tar. Holes are drilled on four corners of the sheet/inside the cement wall for expansion fasteners which are fixed by screwing in the nail with 4"-5" long steel wire tied on its head. The wire hangs out of the sheet to be used for tightening of wire net fixed on top of the insulation. The layer of cement plaster is then applied (2 cm) on top of this and given a smooth finish. Bituminous paint is applied on cement plaster as a vapour barrier. The painting can be avoided in cropping rooms if the cook out is not done by steam. This wall will be good enough to give a K value of 0.5-0.6 kcal/m²h, even lesser and will facilitate proper control of climate inside the cropping room.

iii. Roof

The roof is made of RCC (1 : 2 : 4) 12-15 cm thick. The inside is given a cement plaster finish for application of insulation (as explained for the wall). The roof on the outside is protected by tarring it on top, followed by 10 cm thick loose soil, 5 cm thick mud capping and finally the tiles. This will protect the roof from weathering effects of rain and will ensure longer life of insulation and prevent seepage of moisture into the room in rainy season. In hilly areas with a high rainfall index, slanting GI sheet roof over the insulated RCC roof will be excellent and in that case mud capping/tiling of the roof is not required.

iv. Doors/vents

The doors of the bulk chamber and the cropping room are made of wood or angle iron frame covered on inside and outside with aluminum sheets/GI sheets with insulation of 5-7 cm in the middle. The doors will have a rubber gasket lined on inner periphery so that the door becomes air tight when closed. The door will operate on hinges, with a strong locking latch for opening and closing of the door. The exhaust vents are fitted with wire net, louvers and insulated lids. The louvers allow the CO₂ laden air to exhaust under positive pressure created by the blower inside the air handling unit.



v. Lighting arrangement

There should be a provision for tube lights and a mobile strong light for inspection in each cropping room. The tube lights should be protected with water proof housing. The tube lights should be fitted on all the walls vertically at various heights to facilitate lighting of all beds. There should be provision for a few electric points (5 and 15 Amp.) for operation of water spraying equipment and CO₂ measuring instruments.

vi. Water connection and sewers

One clean water pipe line (1" or 1.25") with tullu pump installed to it for delivering clean water for spraying should be provided in each room. Underground drainage line for carrying the washings from the room and wash basin discharge should be laid before construction of the building. This waste water line should be connected to the common sewer. In H.D. polythene cropping rooms, sunkun traps on the floor for fresh water and drainage water are provided inside the growing house with each trap of 1' x 1' x 1' dimension fitted with an iron lid on top. It is desirable to lay underground drainage in the central gallery in advance of erecting the structure for carrying away the waste water/ washings from the cropping rooms.

vii. Gallery

The gallery between the rows of cropping rooms should be wide, (12-15 ft) to allow efficient performance of various operations. The height of the gallery should be same as for the growing rooms alternatively it may be about 8' with a false ceiling, leaving another 5 ft above for pipeline and space for AHUs.

viii. Racks

Racks are made up of the angle iron for horizontal and vertical support with iron mesh strips used for the shelves for housing compost. Length (vertical axis) of the racks is generally made up of 5 cm thick angle while horizontal support is made up of 3.5-4 cm thick. Width of the each shelf on the racks should not be more than 135 cm in any case as width more than that creates hindrance in performing various operations during cropping and most important of that is harvesting. Cultivation can be done in bags or in shelved beds. Five to seven rows of shelves (depending on height of the room) can be provided one above the

other in the racks keeping a minimum distance of 60 cm in between. This distance can slightly be narrowed down if cultivation is employed in shelved beds. In such a case all the four sides of the shelf should be provided with 15- 20 cm high iron sheets for housing the compost in the beds. If more than 5 shelves on each rack are kept in the room than there should be provision of trolley running in between two rows of racks just above the fourth shelf for carrying out the various operations. Depth of the compost in shelves is generally kept at 15-20 cm while bags can be filled up to the maximum height of 30 cm. A room of standard size (60 x 17 x 12 ft) can accommodate 2 rows of racks each 4.5 ft. (135 cm wide). This will absorb 9 ft (270 cm) of the room and the rest 8ft can be used to have one central path of 3 ft. and 2 side paths of 2.5 ft. Length of each rack would be 52-55 ft.



ix. Air handling unit

This unit is employed for creating proper weather inside the growing room specific to white button mushroom. Air handling unit is generally installed in each room at the top of the door, which is made up of aluminum or G.I. Sheets. In certain cases it can also be placed on the top of the floor of the growing room or in the corridor. Indirect cooling of air through chilled water (5-6°C) is generally employed in mushroom cultivation. Mushroom generally requires 225 m³ of air per hour per ton of compost. To meet this requirement a high speed centrifugal fan of required capacity having working pressure around 50 mm WG is generally mounted in the body of AHU. Alternatively if the capacity of the growing room is to accommodate around 20-25 tons of compost, then a fresh air fan of 600 mm dia of low pressure can also be chosen for this purpose, but in such case a booster fan of 375 mm dia will also required to be mounted in AHU for extracting fresh air from outside. In AHU cooling coils, humidifiers, heaters, eliminators and other components of AHU are mounted on the back of the supply air fan. Cooling coils are generally connected to the chilling unit via insulated ducts, which supply chilled water at 5-6°C to these coils. This water is generally chilled in an insulated tank or by cooling unit comprising of a compressor, condenser, evaporator and a cooling tower. Heating unit of AHU can employ strip heaters or steam through a low-pressure boiler. Humidifiers can use free steam from the boiler to generate required humidity in combination with air pressure or can employ fine jets, which produce fine mist of water in the humidifier section of the AHU. PVC eliminators, eliminate the free water going inside the growing room. Booster fan in combination with supply air fan supplies fresh air inside the AHU through fresh air dampers. Since fresh air coming from outside atmosphere may contain fungal spores, which may contaminate the crop, this air is generally passed through pre filters and a HDPE micro filter section (2-5 µm). The AHU has a mixing chamber with recycling dampers, which can regulate supply of fresh air or room air inside the growing room. Out let of the AHU is connected to the distribution duct in the growing room, which is generally made up of PVC sheeting having its end mouth closed. It hangs below the ceiling in the central corridor of the room. This duct has ports (5 cm dia) facing downward at a distance of around 50 cm each. When the air is blown inside the room via AHU a positive pressure is created and CO₂ laden air of the

growing room is expelled in the atmosphere through an outlet. In such cases back vents are not provided in the growing rooms. Alternatively AHU can be so fabricated having provision to exhaust CO₂ laden air of the growing room in the atmosphere through an out let. In such cases back vents are not provided in the growing rooms. Central cooling unit can employ ammonia, Freon or vapour absorption machine (VAM) for cooling purpose. If size and capacity of growing unit is small, say 250 MT per annum employing around 12 rooms then cooling employing evaporator, inside the AHU can also be chosen. In such a case each AHU will be a self contained cooling unit, employing compressor, condenser and an evaporator. This unit will also have heating and humidifying arrangements.

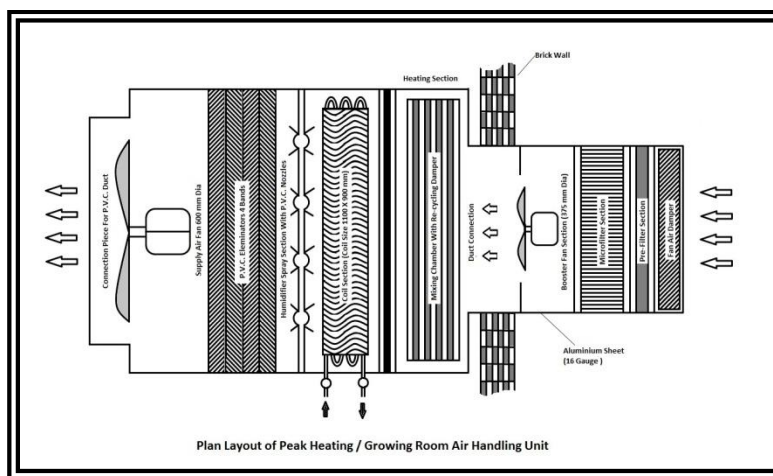


Fig 4. Schematic diagram of AHU

Crop Management

Button mushroom cultivation has two major components, composting (preparation of substrate/compost), and the crop management, (raising of mushroom crop). The substrate preparation has undergone scores of innovations/improvements suiting environment protection laws in many developed countries. At the same time, casing medium has also been standardized with use of peat and its alternative materials (FYM, Spent Mushroom Compost and Coir Pith) with prime objective to improve productivity and quality of mushrooms. Similarly, the crop management techniques have also been improved upon to harvest highest possible mushroom yield over a shortest period of time. All the operations/applications done after completion of composting are handled under the head crop management. These include:

- A. Agronomic crop management
- B. Environmental crop management

A. Agronomic Crop Management

Agronomic crop management deals with the compost quantity to be filled per m² bed area, moisture content of compost, spawning method employed, compost thickness in a bed or bag, casing application and thickness, watering regimes employed, harvesting of crop and after care, pest management, hygiene maintenance and so on. However, more important among these are

1. Spawning and spawn run
2. Casing materials, casing treatments, casing application, case run and pinhead formation

1. Spawning and spawn run The steps involved are



- Good quality compost with temperature of 25°C
- Mixing of grain based spawn (@ 0.5-0.7% of wet compost weight) under clean conditions (i.e. with clean hands and pre-sterilized area)
- Filling of spawned compost into polythene bags (12-15" depth) or beds (6-8" depth)
- Little compressing and levelling of spawned compost
- Loosely closing the mouth of polythene bags filled with spawned compost (Covering with a clean newspaper / plastic sheet if filled in trays/shelves)
- Shifting the compost filled bags in cropping rooms with a temperature of $23 \pm 1^\circ\text{C}$ (air temp.), RH of 95% and high CO₂ conc. (1.0-1.5% strain dependent), and keeping the bags under above conditions for 12-14 days
- Completion of spawn run (change of dark brown compost mass in to light brown colour)

Precautions

- Use of fresh pure culture spawn
- Spawning under clean conditions (preferably under positive pressure created using bacterial filters before inlet fans and air curtains at doors)
- Proper treatment of spawning area and tools with formalin, and cleaning of hands with dettol
- Maintaining good hygienic conditions during spawning by keeping all the doors/ windows closed

2. Casing and case run Casing is a 3-4 cm thick layer of soil applied on top of spawn run compost and is a pre-requisite for fructification in *A. bisporus*.

a. Casing materials Earlier sub-soil material or organic matter rich soils were used as casing in button mushroom cultivation. Presently peat is the most desirable casing material used world wide with excellent mushroom yields and superior fruit body quality. However, peat is not available in India. The other alternative recommended materials are,

- Well decomposed Farm Yard Manure (FYM) preferably two years old
- Well decomposed Spent Mushroom Compost (SMC) (two years old anaerobically decomposed)
- Composted coir pith (coir industry waste) (well decomposed & water leached)
- 1:1, 2:1 and 1:2, v/v of well decomposed FYM and SMC
- 1:1, v/v of decomposed FYM or SMC with composted coir pith
- Decomposed powdered bark of some forest trees
- Paper industry waste
- Burnt rice husk is also in use along with decomposed FYM (2:1, v/v) in seasonal cultivation of button mushroom in Hayrana and Punjab with reasonable success

b. Quality of casing materials

- Soft texture
- Light weight
- High water holding capacity
- High porosity
- Deficient in available form of C and N
- Neutral pH (7.0 – 7.5)
- Low conductivity (400-600 μ moh)

c. Casing treatment Casing material should be treated properly before its application on the spawn run compost and the steps involved are:

- Make a heap of casing material
- Wet it up to 50-60% water holding capacity
- Fill in trays and shift them to pasteurization chamber

- Steam pasteurization at 60-65°C for 6-8 hours
- Auto-Cooling

Alternatively,

- Make a heap of casing material on a cemented platform
- Wet it up to 50-60% water holding capacity
- Drench the wet casing with formalin @ 1 litre/m³ (40% formaldehyde) by mixing with shovel
- Cover it with polythene sheet and seal the outer periphery thereafter by pouring sand/soil on outside margin
- Keep the material for 24-48 hours in sun for fumigation effect
- Remove the cover after 48 h and expose the material to open air and sunlight by spreading over with clean tools and permitting the formalin fumes to escape in to air for 2-3 days before it is used as casing (formalin treatment effect decreases at low temperature due to inadequate fumigation)

d. Casing application

- Unfold the fully spawn run bag and make the top surface even by gentle pressing with hands
Light spray of water on spawn run compost
- Application of 4-5 cm thick layer of casing uniformly using iron rings of 4 cm height or wooden blocks
- Water spray in instalments immediately after casing application

Precautions

- Casing material should not be sieved but used as such with clumps, which permits more air spaces in casing
- Top casing surface should have small mounts and valleys
- Care should be taken to prevent re-infection of the casing materials
- Store casing material in a sterilized /clean room before use in polythene bags or synthetic cloth bags
- Apply water to casing in a few installments so that water does not run into spawn run compost

e. Case run and pinhead formation

Case run is done at a temperature of $24 \pm 1^\circ\text{C}$, RH-95% and $\text{CO}_2 > 7500$ ppm (strain dependent) for about one week. There is no requirement for fresh air introduction during case run. It is considered complete when mycelia come in the valleys of casing layer. After case run, the environmental conditions are changed by bringing down the temperature to 15-17°C (air), RH to 85% and CO_2 to 800-1000 ppm (strain dependent) by opening of the fresh air ventilation and exhausting CO_2 . This change in environmental parameters induces pinhead formation in 3-4 days (strain dependent) time. The pinheads develop into solid button sized mushrooms in another 3-4 days. At this stage, the air inside the cropping room is changed 4-6 times in an hour to maintain appropriate CO_2 conc. as CO_2 production is at its peak during first flush (actually peaks at case run).

3. Supplementation

Supplementation with protein rich supplements such as cotton seed meal, soybean meal, alfa-alfa meal, feather meal, etc. has been found to increase the mushroom yield. Supplementation can either be done at spawning or after spawn run before casing. The later is more useful. Supplement is first grounded coarsely and denatured by treating with 5000 ppm formalin and before its mixing in compost. The practice normally increases the temperature of compost by 4-5°C and if done at the time of spawning or in poor quality compost, it results in killing of mushroom mycelium or increased incidence of moulds. If these problems are overcome supplementation can give 20-25% enhanced yield. Supplementation at casing in spawn run compost also helps in early and higher mushroom yield.

4. Ruffling

Ruffling of compost on completion of spawn run is done just before casing. This practice is particularly useful for round the year cropping when 5-6 crops are taken per year and cropping period is reduced to about 4 weeks, as this practice helps in exhaustion of compost earlier than normal. Ruffling of casing after a 3-4 days or so after casing is done by some growers to get uniform pinning.

5. Watering

Mushroom contains nearly 90% water and that gives us an idea how water is important for the crop. Mycelium gets water from compost during spawn run and compost + casing during case run and from casing during fruit body formation. Water level in casing is maintained in 2 ways. One way is by its regular spray when pinheads are pea sized and then by maintaining RH at 80-85% during cropping. If one of the factors, (water spraying and RH) during cropping is disturbed, it will affect crop productivity. Low RH during cropping will result in drying of beds, lightweight mushrooms, discoloration of mushrooms and crop losses. Drying of casing will seal the casing medium resulting in mat formation, which becomes impervious to water, and results in tremendous crop losses. Water has to be replenished in casing to accommodate the water losses from casing due to mushroom growth and evaporation. Lesser the water loss to room air, better it is. Bed moisture and RH are although two different factors, but are interdependent. Water spraying on mushroom beds at pin breaks should be avoided. The casing should be wet enough when fresh air is brought in and room temperature lowered. The wetness should be sustained till pin heads become pea sized, and that is the stage when bed will require additional watering to allow pea-sized pins to develop into button sized mushrooms. Watering to beds requires monitoring at each stage. RH in the cropping room is monitored by using dry & wet bulb thermometers. Two ordinary stem thermometers are put in the cropping room, placing one in the casing/compost bed and one hanging in the air nearby (few cm apart). Bed temperature is 1-2°C higher than air temperature. Computer control of AHU ensures application of cropping parameters with precision during spawn run, case run and cropping. The water used for irrigation (spraying) on mushroom beds should be clean, neutral in pH and free from salts, heavy metals and other impurities. Water good enough for drinking/watering for vegetables/field crops is also good for mushroom cultivation. It is desirable to test the quality of water before the mushroom growing is started at a particular site.

6. Harvesting and after care

Mushrooms with 4-5 cm dia., with hard pileus and closed veil are ready for the harvest. Mushrooms are harvested by holding them between forefinger and thumb, and rotating in clockwise/anticlockwise direction. The soiled stem portion is cut with sharp edged knife and mushrooms are collected grade-wise in baskets. Dropping of the stem cuttings on the floor or the bed should be avoided, as these will promote the growth of undesirable microorganisms. Cleaning of mushroom beds and floor is recommended after each crop harvest. Fresh casing is applied at places from where mushrooms have been removed. Water is sprayed at the rate the mushrooms have been harvested, i.e. for every kg of mushroom harvested 1 litre of water is added after harvesting. Damaged pins/ mushrooms, if any, are also to be removed from the bed manually. If bunching of mushrooms is observed, then there is a need to address the climate controls for creation of optimal environmental conditions during pinhead formation. Mushrooms after harvest are graded, packed in PP bags/card board boxes and preferably chilled at 4°C for 6-8 hours before sending to the market. The pre-market chilling enhances the shelf life of mushrooms. While harvesting care should be taken to keep the pileus free from casing soil, as it stains the mushrooms. Washing of mushrooms to make them extra white for increased acceptability in the market is undesirable, especially with Potassium metabisulphite solution. Unwashed mushrooms stay fresh for a longer period. Mushrooms should be handled carefully, and not bruised during the harvesting operation. Bruising will damage the mushroom tissue, which will turn the pileus dark/ pink on exposure to air. While packaging mushrooms in PP bag one should not forget to make a small hole (0.2 mm), as it will prevent the development of aflatoxins in transit or storage. Button mushroom can be stored at 4°C for a few days without any deterioration in its

quality but it is desirable to consume/market fresh mushrooms. Since button mushroom has a very short shelf life and it cannot be stored for longer periods, hence it requires processing for long storage. Mushrooms are best preserved in brine solution after blanching, either in cans or jars. The properly processed mushrooms stay in good condition for over a period of 1 year. It is possible to transport canned mushrooms over longer distances without any deterioration in their quality. But fresh mushrooms can only be transported short distances in refrigerated vans/by air to reach up to a remunerative market.

B. Environmental Crop Management

Mushroom is an indoor crop, raised in cropping rooms with simulated environmental conditions suiting to a particular mushroom. Hence management of crop environment becomes utmost important. It includes the temperature, RH, CO₂ concentration, air speed/ evaporation rate over crop beds, air changes in the room/oxygen availability and other such factors, which directly influence crop productivity. The environment management in the cropping room includes addressing of the following factors: 1. Temperature, 2. Relative humidity (RH), 3. CO₂ concentration

1. Temperature

Temperature in the room has two areas for monitoring i.e., air temperature and bed temperature. Temperature has direct bearing on crop productivity in synergy with other factors like RH and CO₂ /O₂ conc. in the cropping room. The bed temperature in the cropping room is directly influenced by the air temperature, so it is the air temperature that has to be addressed. The air temperature inside the room can be manipulated with use of cooling/heating coils in an Air Handling Unit (AHU) installed inside or outside the cropping room for climate control. An independent AHU is desirable for each cropping room. The AHU inside contains a set of cooling coils, heating coils, RH fogging jets and a centrifugal blower fan for blowing the conditioned air into the cropping room. The AHU is generally installed on top of the entry door and is joined with a recirculating duct from inside the cropping room. The cooling coils are fed with chilled water from the chiller, while the heating coils are fed with steam from boiler and fogging jets get water from trough placed at the bottom of the AHU by a small pump. The cooling requirement will depend upon compost quantity fed inside the room, outside prevailing temperature, insulation on the walls, etc. The blower fan blows the conditioned air into the room. The fresh air into the room goes in via AHU through a control valve, and during most of the crop raising period fresh air valve is placed at 20-30% and re-circulating at 70-80%. During spawn run the entire air is re-circulated (100%) and no fresh air entry is required.

a. Spawn run

For spawn run air temperature of $23 \pm 1^{\circ}\text{C}$ is maintained inside the cropping room, which corresponds to bed temperature of 24-25°C (1-2°C higher than air temperature). During this phase, the fresh air valve is closed and entire air is recirculated, allowing the carbon dioxide to accumulate to the level of 15000 ppm, desirable for quick spawn run. Higher concentration of CO₂ accelerates the spawn run/vegetative growth of the mushroom. Any increase or decrease in temperature effects the CO₂ production of the compost and the RH of the room. With increase in temperature, RH will tend to fall, and just vice versa with decrease in temperature. The properly insulated room will ensure uniform temperature inside the cropping room at every stage of crop growth. The heat from the cropping room is removed via cooling coils fitted inside the AHU.

b. Case run

The environmental conditions suitable for spawn run, are suitable for case run as well. The same conditions, as for spawn run will be continued for next 7 days for case run, i.e., temperature of $23 \pm 1^{\circ}\text{C}$ in the air and 24-25°C in the bed. The RH/CO₂ will also be same as for spawn run. Under aforesaid conditions the case run will be completed within one week, and at the same time the mycelium is observed in the casing valleys. Valleys are the areas between the peaks as can be seen on top of casing.

The CO₂ conc. and RH should also be maintained within the optimum range for quick and effective case run.

c. Cropping

After completion of case run, cooling inside the room is enhanced to bring the air temp. down to 15-17°C in the room within 2-3 days time. Simultaneously, the fresh air vent is opened to 30% and rest of the air is recirculated (70%). This brings down the CO₂ conc. inside the room to 800 to 1000 ppm, desired for pinhead formation. Likewise, the RH is also reduced to 85% from 95%. This facilitates pinhead formation on the casing within a week's time. The pinheads grow into full button sized mushrooms in another 3- 4 days. At this stage fresh air can be slightly reduced to achieve 1000-1500 ppm CO₂ concentration. The environment parameters are maintained as above during entire period of cropping. Since the temperature has influence on RH and CO₂ production from compost hence should be manipulated, keeping in mind its effect on other two factors. All the three parameters work in synergy with each other to induce pinning. The pinning will be affected adversely if any of these factors is not in its optimal range. High temperature for a long period of time during cropping will lead to sealing of casing, and will result in stopping of pinhead formation. The mycelium will continue growing in vegetative phase and will seal the casing, making it impervious to water, thus resulting in serious yield losses. The desired temperature in cropping room can be maintained with good precision by the use of sensors and controlling devices attached to cooling/heating coil inlets fitted inside the AHU. These devices are easily available and are effective in temperature control in the cropping room.

2. Relative humidity

Relative Humidity (RH) is the ratio/proportion between absolute humidity (AH) and saturation point of humidity (SPH) at a given temperature, expressed in percentage. Absolute humidity is number of grams of water vapours contained in a cubic meter of air at a given temperature. Saturation point of humidity is the maximum number of grams of water vapours feasible in a cubic meter of air at a given temperature. Relative humidity (RH) of 85% is necessary for obtaining highest pin head formation in synergy with other factors like temperature and CO₂ concentration. RH of 85% permits slow evaporation of water from the crop bed to air in the cropping room and thereby facilitating the upward movement of nutrients in the compost. This exchange of air facilitates loss of CO₂ + heat into the air, necessary for healthy pin head development and crop productivity. In the event of RH falling below 85% inside the cropping room, more moisture from the crop bed will be withdrawn resulting in drying of the casing layer. This will seal the casing and result in crop losses. Lower RH in the room will be indicated by bed temperature falling below the air temperature, an undesirable situation to be avoided at any cost. Under normal circumstances the bed temperature is always higher by 1-2°C than air temperature for development of a healthy crop of mushrooms. For round the clock monitoring of RH, monitoring of the bed and air temperature inside the room is desirable. The incoming air should be humidified enough to prevent loss of moisture from the crop beds. Evaporation of moisture from crop beds has to be taken into consideration for calculating the g of water vapours required per m³ air in a room for maintaining the required RH for cropping. Air in a cropping room contains 9.6 g water vapours per m³ of air at 14°C (A), the saturation point of humidity at 14°C is 12 g/m³ (S). The RH of the room air will be $A/S \times 100 = 9.6/12 \times 100 = 80\%$. The ultimate expression is the quantity of water vapours contained per m³ of the air space of the room at a given temperature. 31 g of water vapours gets evaporated from 1 m² bed area at 17°C/85% RH/hour. The change in room temperature will alter the RH in the room. Use of RH sensors with cut off/starting devices for recording and maintenance of RH in a cropping room is very useful. The sensors will control the fogging jets in the AHU as per the requirement in the room. For obtaining a temperature of 17°C and RH of 85% in the cropping room, air temperature is brought down to 14°C at exit point of AHU with 100% RH. The air on reaching the crop bed will receive some heat from crop bed and raise the air temperature to 17°C with RH automatically falling to 85%.

3. Carbon dioxide

Carbon dioxide concentration is the third important factor in management of environment inside the cropping room. CO₂ is produced by actively growing microorganisms in compost during spawn run, case run and by mushroom mycelia and mushrooms during entire cropping cycle. During spawn run, higher concentration of CO₂ is desirable, which helps in quick and quality spawn run. For spawn run, CO₂ concentration between 10000-15000 ppm is desirable (strain dependent) and it helps in quick spawn run in compost. Higher concentration of CO₂ is also desirable during case run. For pinning and cropping, the CO₂ concentration is lowered around ambient (800-1000 ppm). CO₂ concentration up to 1500 ppm is maintained during pinning & cropping, and this is done by venting/opening of fresh air duct to bring in oxygen and exhaust of CO₂ from exhaust vents under positive pressure. The opening of vent will bring in fresh air, which is conditioned in AHU (heated or cooled/humidified) and then blown into the cropping room via ducts. The CO₂ gets mixed up with the fresh air and is carried under positive pressure towards the exhaust vent and finally exhausted. This also facilitates the exhaust of heat alongwith the CO₂ from the room air. The heat is removed via cooling coils after the room air gets into the AHU via recirculating duct. During air circulation, recommended air speed over the crop beds is 15cm/sec. Ensure that the desired air movement is there in the central shelf in the middle row. This can be checked with the help of a burning incense stick, which will indicate the direction of air movement in the cropping room. Higher concentration of CO₂ during pinning can seal the casing or produce onion shaped mushrooms with a bulbous base & a small cap. During development from pinhead to button sized mushroom, higher concentration of CO₂ will lead to long stiped mushrooms with a small cap (opened), which reduces the crop yields. By gentle movement of air over the crop beds, the CO₂ is carried away from the crop canopy, thus saving the bad effect of CO₂ trapped between the mushrooms in the crop canopy. To ensure healthy crop production, about 6 air changes per hour are recommended from the venting time to completion of first 2 flushes. During this period, CO₂ production is highest (10 g/h/m²) and it requires to be removed at a faster rate. Along with CO₂, heat is also produced @ 10W per hour from one m² bed area at 17°C and 88% RH. In subsequent flushes, 4 air changes per hour are sufficient to maintain right O₂ content in the cropping room (about 16%). During first two flushes fresh air vent is opened to 30% entry and 70% recirculation, and in subsequent flushes the fresh air vent is put at 20% and recirculation at 80%. Use 2 µm mesh filters on fresh air entry points into the cropping room to restrict the entry of diseases/competitor mould spores. The CO₂ after mixing with the room air, gets exhausted under positive pressure from exhaust vents, thereby helping in heat + CO₂ removal from the room. Maintenance of right combination of casing moisture (about 50 ± 2%), CO₂ concentration, RH and temperature at pinning stage of crop growth helps in obtaining a heavy pin set, thus resulting in a luxurious crop growth and excellent yield of mushrooms. If onion sized mushrooms/drum sticks are observed, correct air circulation for effective CO₂ removal from crop beds is required. Lack of air movement and accumulation of CO₂ creates this type of situation. Long stemmed mushrooms are again the outcome of CO₂ accumulation in the air around crop canopy due to faulty air movement/air circulation inside the cropping room.

C. Airing Procedure for Fruiting Venting or opening of fresh air for induction of fruiting after case run is a critical phase in mushroom growing. Whether to cool first or bring in fresh air first is a question bothering commercial mushroom growers. The airing is done suiting a particular situation, whether one wants to have a heavy first flush followed by moderate flushes later or equally spaced flushes. The airing accordingly is handled under 3 heads: 1. Soft airing 2. Moderate airing 3. Severe airing

1. Soft airing

Soft airing means that we will have severe restriction on venting to get smaller flushes suiting to market demand and the air is opened slowly. The growing parameters to be manipulated for soft airing are listed below: Air temperature : 19°C in 48 hours, 17°C in 72 hours, Compost temperature : 21°C in 96 hours, CO₂ concentration 4000 ppm in 48 hours, 2000 ppm next 24 hours, 1000 ppm after 72 hours, RH 98% to 92% in 48 hours.

2. Moderate airing

Moderate airing means that we will have some restriction on airing/venting to get well spaced flushes of moderate levels. The growing parameters to be manipulated for moderate airing are listed as under: Air temperature 17°C in 24 hours 20°C in 72 hours, Compost temperature 20°C in 72 hours, CO₂ concentration 2000-2500 ppm in 24 hours, Less than 1000 ppm in 48 hours, RH 98% to 92% in 24 hours.

3. Severe airing

Severe airing is done to obtain a heavy first flush and no restriction is put on airing. This results in heavy pin set and large first flush, followed by smaller subsequent flushes. The growing parameters to be manipulated for severe airing are listed below: Air temperature 15°C as soon as possible, Compost temperature 20°C in 48 hours, CO₂ concentration, Less than 1000 ppm in 12 hours, RH 98% to 90% in 12 hours.

Action Points

- a. Observe strict hygiene throughout the farm
- b. Ensure that the temperature during peak heat is satisfactory
- c. Make sure that casing ingredients are stored and mixed in clean area and casing is properly pasteurized
- d. Make sure that all spent compost is removed from the farm
- e. Properly clean the cropping rooms after every crop

4. PROCESSING

During the past 5 years, the consumption of mushrooms has grown 15 times. Besides the sizable domestic market, which is underfed, there is great demand for mushrooms in USA, France, Germany, Canada, Italy and UK. Besides this, fresh market in the gulf remains untapped. China which is the largest exporter of mushrooms to the American and European countries is facing anti dumping duties on its products. Further Chinese mushrooms are not available throughout the year, and hence it is the right time that India enters billion-dollar global mushroom market to earn valuable foreign exchange for the country. Since the promoters are already in the processing business including canning, they are seeing tremendous potential in this field. They are unable to meet the demand of canned mushrooms as the fresh mushrooms are not available easily and if available they have to pay very heavy price for the same thereby eroding the profits. The demand for mushrooms, domestic as well as international is rising at a phenomenal speed. World production of mushrooms was about 12.2 million tons in the year 2002 and China remains the main producer and exporter of mushrooms. India is roughly producing around 1,81,000 tons of mushrooms annually of which 60,000 tons is produced by a single unit the Agro Dutch Foods Lalru, Punjab, which boasts of the single largest producer and exporter of mushrooms in the world. Besides this very big unit there are many other small white button mushroom units in Punjab, Haryana, Uttarakhand, Maharashtra, Gujarat, etc cultivating this mushroom all the year round and are running successfully. These units are located in Phagwara, Jalandhar, Bhatinda, Banga, Bannore, Haridwar, Dehradun, Pune, Nasik, Badnagar, etc. In the state of H.P., units located at Poanta sahib and Nalagrah are doing exceedingly well and are in for expansion. In Uttarakhand, M/s Flex Foods is doing very good and producing around 2000 tons of button mushroom. The promoters don't foresee any problem in marketing their produce.

Now with adoption of latest technology of mushroom production under controlled environmental conditions, it is possible to grow high qualities of mushrooms throughout the year to meet the domestic and international demand. The promoters have undertaken the market surveys and made inquiries regarding the demand for mushrooms. Besides the big demand in the countries mentioned above there is a fast mushroom market developing in the gulf countries. Domestic market is also expanding at phenomenal rate, which is reflected in the increase in the production of mushrooms. Most important of all for this project is ever increasing demand and lucrative prices for canned mushrooms in India and abroad. Our per capita of mushrooms consumption of the mushrooms is the lowest in the world, which is 60-80 g

against the 3 kg in the developed countries. This poor consumption is mainly due to non-availability of mushrooms in most part of the country for most of the year. As such no difficulty in marketing of mushrooms will be experienced.

Freeze dried and canned mushroom is high value low volume products, which may be marketed in domestic as well as international market for a longer duration against the fresh mushroom. The postharvest shelf life of these products is quite high helping the processors to get remunerative returns for their produce. Further, the processing units will also help to collect the produce from the local producers helping them to avoid the distress sale. These units will also provide job opportunities for the rural youth, which is the need of the hour.

WHY MUSHROOM PROCESSING

1. High quality mushroom
2. Longer shelf life
3. To reduce distress sale of mushroom growers
4. Assured market, continuing revenue and sound profitability
5. Labour intensive providing gainful employment
6. Foreign exchange earner through exports

Various levels of technologies are available for processing of mushroom right from cabinet drying, freeze drying, dipping and canning. Freeze drying, also known as lyophilization or cryo-desiccation, is a low temperature dehydration process that involves freezing the product, lowering pressure, then removing the ice by sublimation. This is in contrast to dehydration by most conventional methods that evaporate water using heat. Freeze drying results in a high quality product because of the low temperature used in processing. The original shape of the product is maintained and quality of the rehydrated product is excellent. Primary applications of freeze drying include biological (e.g., bacteria and yeasts), biomedical (e.g., surgical transplants), food processing (e.g., vegetable, coffee, mushroom, etc) for preservation. The primary purpose of freeze drying within the food industry is to extend the shelf-life of the food while maintaining the quality. Freeze-drying is known to result in the highest quality of foods amongst all drying techniques because structural integrity is maintained along with preservation of flavors. Because freeze drying is expensive, it is used mainly with high-value products. Examples of high-value freeze-dried products are seasonal fruits and vegetables because of their limited availability, coffee, mushrooms and foods used for military rations, astronauts/cosmonauts, and/or hikers.

The studies have been undertaken on postharvest management packaging and drying of different mushrooms to extend the shelf life which is otherwise very low.

1. Packaging and storage

Button mushrooms

Washing

Washing in KMS (0.20%) significantly improved the whiteness of button mushroom with lesser rate of browning during storage than lower concentration (0.05%). Blanching of mushrooms caused significantly browning besides 30-40% loss in weight while washing treatment increased the weight by 8%. In the areas having higher temperature, the KMS concentration should be used only upto 0.05% or lower.

Pre-treatments

Out of different concentrations of sodium citrate (625, 1250, 2500 and 5000 ppm) and EDTA (125, 250, 500, 1000 ppm) in postharvest treatment of button mushroom revealed that washing with 125 ppm and 250 ppm EDTA significantly improved the quality and shelf life of button mushroom stored at ambient temperature for 48 hours and six days at refrigerated conditions. Further, 125 ppm EDTA was found

better than 0.05% KMS for retention of quality and shelf life of button mushroom at refrigerated conditions. The washing treatment with 125 ppm EDTA reduced the total surface bacteria present on the fruit body.

Among different edible coatings tried in button mushroom and stored at ambient and refrigerated conditions revealed 12 days shelf life with 0.5% ascorbic acid in carboxymethyl cellulose (CMC) at refrigerated conditions.

Packaging

Button mushroom stored in non-perforated polyethylene bags revealed a storage life of 3-4 days at 5°C and 2 days at 10°C. A significant decrease in vitamin C, total protein, phenols, total soluble sugars and non-reducing sugars recorded during 4 days storage being more pronounced at 15 and 10°C than 5°C. It was suggested not to perforate the poly-packs when to be stored at ambient temperature.

In modified atmosphere packaging (MAP) of button mushroom it was found that PP (Polypropylene) and PE (Polyethylene) bags are highly permeable to O₂ and CO₂ gases and very little MAP was created within the bags. However, the punnets over wrapped with PVC film established the gaseous equilibrium of O₂/CO₂ within 2 hrs at ambient temperature. In MAP of button mushroom in PET jars diffusion channel (3 mm dia and 15 cm length) was found to be the best method to prolong the shelf life of button mushroom upto 8 days in ambient storage (18±1°C). In another experiment mushroom packed in 100 gauge PE and PP having 80% N₂ and 15% CO₂ revealed that 100 gauge PP bags were found good upto 4 days.

The treated white button mushroom was stored in different capacity PE (150 gauge) at ambient and low temperature. It was observed that the mushrooms stored in 200 g and 400 g capacity had a better shelf life both at ambient and low temperature compared to higher capacity (600, 800 and 1000 g). The white button mushroom was treated with 0.2% citric acid for 5 minutes followed by packing in different capacity PE bags at ambient (18-20°C) and low temperature (4-6°C). It was observed that the mushroom has a shelf life of 6 days at ambient temperature as compared to 10 days at low temperature.

The white button mushroom (*Agaricus bisporus*) var. NBS-5 were stored in different packaging material viz., Polyethylene (150 gauge), jute, bubble wrap, non-absorbent cotton, corrugated fibre board (CFB), brown paper, cotton, newspaper, punnet along with control (without packing) at ambient (25°C) and low temperature (4-6°C) in 400 gm capacity. Out of these, Polyethylene (150 gauge), plastic punnets with PVC covering and bubble wrap packaging materials were found most suitable for better quality retention and shelf life extension of white button mushrooms during storage.

Oyster Mushrooms

Significant increase in shelf life in oyster mushroom (*Pleurotus florida*) could be achieved by pre-cooling the produce at 5°C for 2 hrs before packing in trays for storage at ambient temperature. Perforation of packs though resulted in moisture loss but toughens the surface texture preventing further loss of moisture and increasing the shelf life.

Oyster mushroom (*Pleurotus florida*) was stored in different capacities of PE bags at ambient (18-20°C) and low temperature (4-6°C). It was observed a shelf life of 5 days at ambient temperature having maximum PLW in 600 and 800 g bags compared to 200 and 400 g whereas the shelf life was more than 20 days at low temperature storage conditions.

Paddy straw mushrooms

The paddy straw mushroom packed in PP bags (open condition) was found good upto 2 days irrespective of storage conditions. In the storage of *Volvariella bombycina* at refrigerated and ambient conditions in transparent plastic trays with two holes revealed rate of loss in weight of less than 1% per day in refrigerated condition compared to 11.23 to 14.43% per day at ambient conditions. The keeping quality of fruit bodies stored under refrigerated conditions (4±2°C) was almost at par with fresh fruit bodies even after their storage for 7 days whereas the fruit bodies at ambient (20±4°C) conditions were acceptable only for one day.

2. Drying and dehydration

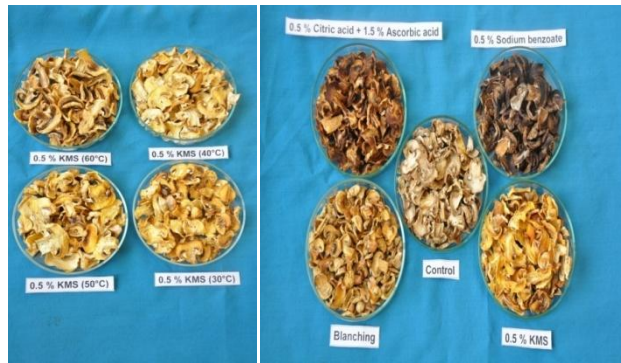
Rehydration of dried slices of button mushroom was significantly better than that of dried whole. Complete slices (with pileus+stipe) may be used for culinary preparation after rehydration. Small irregular pieces may be powdered for use in mushroom soup and also as flavouring agent. Drying of oyster mushroom in hot air oven at 55°C gave the best product with regard to texture, color and rehydration compared to sun drying. At higher temperature, the color of the product was more brown than at 55°C. Big sized fruit bodies of oyster showed very poor rehydration and it was extremely poor in stalk portion. Creation of few cuts in stalk and boiling in 0.5% sodium chloride facilitated quicker rehydration. Thick stems should be removed before drying. Partially dried (40%) oyster mushroom gave better snacks without sticking to the drying surface. The sun drying of oyster mushroom followed by micro-wave oven drying gave better quality mushroom chips. Post drying spicing and salting proved better treatment. In drying of button mushroom pre-washing for two minutes in a solution of 0.05% KMS + 0.1% citric acid gave the best product with respect to whiteness.

In osmo-air drying of oyster mushroom it was found that the best quality product in terms of whiteness and high weight recovery was produced from dipping treatment in solution containing 8% NaCl, 0.3% citric acid, 0.5% ascorbic acid, 1% cane sugar and 1000 ppm KMS. In button mushroom, maximum whiteness in the dried slices was obtained by dipping in the solution containing 15% NaCl, 0.3% citric acid, 0.5% ascorbic acid, 1% cane sugar and 1000 ppm KMS. In solar drying of oyster mushroom, out of different treatments 4 ppm Cl_2 was found the best whereas in case of button mushroom it was 0.05% KMS + 0.1% citric acid for better quality of dried product.

Oyster and button mushroom was also dried in cabinet drier at 45°C and it was found that the best treatment is 2 ppm Cl_2 + 0.2% citric acid and 0.05% KMS + 0.1% citric acid in oyster & button mushroom respectively. Oyster mushroom was also found best after treating with 0.05% KMS + 0.1% citric acid and dried in sun whereas, button mushroom was not found suitable for drying in sun. Further, in button mushroom, treatment by dipping in solution containing 20% NaCl, 0.3% citric acid, 0.5% ascorbic acid, 1% cane sugar and 1000 ppm KMS in terms of whiteness and recovery was found best in un-blanchd button mushroom dried in cabinet dryer after keeping in osmotic solutions. In the blanchd button mushroom 15% NaCl, 0.3% citric acid, 0.5% ascorbic acid, 1% cane sugar and 1000 ppm KMS was found best in the osmotic air dried product.

Among various treatments the button mushroom slices dried in cabinet drier at 45, 50 & 55°C revealed that washing solution containing 125 ppm EDTA + 0.1% citric acid and dried at 45°C gave best product than other treatments and higher temperature. At 50 & 55°C, 0.05% KMS was found best for dried button mushroom slices, halves and whole. In freeze drying, button & oyster mushrooms registered the highest rehydration ratio and lowest in case of osmo air dried mushrooms. Further, among various treatment combinations best quality osmo air dried oyster & button mushroom product was prepared by dipping blanchd mushroom in solution containing 8% KCl, 0.3% citric acid, 0.5% ascorbic acid, 1% cane sugar & 1000 ppm KMS. In fluidized bed drying best quality product was found by washing in 0.05% KMS and 0.1% citric acid. Rehydration of dried button mushroom after 50 days storage was found best in control compared to blanchd, steeped and blanchd+steeped.

White button mushroom comprising treatments as control, blanching (5 minutes), KMS (0.5%), citric acid+ascorbic acid (0.5%+1.5%) and unwashed for drying revealed that the drying was fast in blanchd compared to other treatments. The colour was found to be better in the KMS treated mushrooms. In cabinet dryer the drying period was one third to that of sun drying because of continuous heating and drying whereas in sun the material has to keep inside in the evening. At 30°C it took 45 hours for drying of white button mushroom after dipping in 0.5% KMS for 5 minutes whereas the drying period was 30 hours at 40 and 50°C and 25 hours at 60°C. The quality of the dried material was found best at 40 and 50°C.



Freeze Drying

There are four stages in the complete freeze drying process: pretreatment, freezing, primary drying, and secondary drying.

Pre-treatment

Pre-treatment includes any method of treating the product prior to freezing. This may include concentrating the product, formulation revision (i.e., addition of components to increase stability, preserve appearance, and/or improve processing), decreasing a high-vapor-pressure solvent, or increasing the surface area. Food pieces are often IQF treated to make them free flowing prior to freeze drying. In many instances the decision to pre-treat a product is based on theoretical knowledge of freeze-drying and its requirements, or is demanded by cycle time or product quality considerations.

Freezing and annealing

During the freezing stage, the material is cooled below its triple point, the lowest temperature at which the solid, liquid and gas phases of the material can coexist. This ensures that sublimation rather than melting will occur in the following steps. To facilitate faster and more efficient freeze drying, larger ice crystals are preferable. The large ice crystals form a network within the product which promotes faster removal of water vapor during sublimation. To produce larger crystals, the product should be frozen slowly or can be cycled up and down in temperature in a process called annealing. The freezing phase is the most critical in the whole freeze-drying process, as the freezing method can impact the speed of reconstitution, duration of freeze-drying cycle, product stability, and appropriate crystallization. Amorphous materials do not have a eutectic point, but they do have a critical point, below which the product must be maintained to prevent melt-back or collapse during primary and secondary drying. In the case of goods where preservation of structure is required, like food or objects with formerly-living cells, large ice crystals will break the cell walls which can result in increasingly poor texture and loss of nutritive content. In this case, the freezing is done rapidly, in order to lower the material to below its eutectic point quickly, thus avoiding the formation of large ice crystals.[2] Usually, the freezing temperatures are between -50°C (-58°F) and -80°C (-112°F).

Primary drying

During the primary drying phase, the pressure is lowered (to the range of a few millibars), and enough heat is supplied to the material for the ice to sublime. The amount of heat necessary can be calculated using the sublimating molecules' latent heat of sublimation. In this initial drying phase, about 95% of the water in the material is sublimated. This phase may be slow (can be several days in the industry), because, if too much heat is added, the material's structure could be altered.

In this phase, pressure is controlled through the application of partial vacuum. The vacuum speeds up the sublimation, making it useful as a deliberate drying process. Furthermore, a cold condenser chamber and/or condenser plates provide a surface(s) for the water vapour to re-liquify and solidify on.

It is important to note that, in this range of pressure, the heat is brought mainly by conduction or radiation; the convection effect is negligible, due to the low air density.

Secondary drying using a benchtop manifold freeze-drier

The secondary drying phase aims to remove unfrozen water molecules, since the ice was removed in the primary drying phase. This part of the freeze-drying process is governed by the material's adsorption isotherms. In this phase, the temperature is raised higher than in the primary drying phase, and can even be above 0 °C (32 °F), to break any physico-chemical interactions that have formed between the water molecules and the frozen material. Usually the pressure is also lowered in this stage to encourage desorption (typically in the range of microbars, or fractions of a pascal). However, there are products that benefit from increased pressure as well.

After the freeze-drying process is complete, the vacuum is usually broken with an inert gas, such as nitrogen, before the material is sealed. At the end of the operation, the final residual water content in the product is extremely low, around 1% to 4%.

Canning

Canning is the technique by which the mushrooms can be stored for longer periods up to a year and most of the international trade in mushrooms is done in this form. The canning process can be divided into various unit operations namely cleaning, blanching, filling, sterilization, cooling, labeling and packaging. In order to produce good quality canned mushrooms, these should be processed as soon as possible after the harvest. In case a delay is inevitable; mushrooms should be stored at 4 to 5°C till processed. The mushrooms with a stem length of one cm are preferred and are canned whole, sliced and stems and pieces as per demand. Well graded fresh mushrooms white in color, without dark marks on either caps or stems are preferred for canning. Whole mushrooms are washed 3-4 times in cold running water to remove adhering substances. Use of iron free water with 0.1% citric acid prevents discoloration. Thereafter blanching is normally done to inhibit polyphenol oxidase enzymes activity and to inactivate microorganisms. It also removes the gases from the mushroom tissue and reduces bacterial counts. The mushrooms are blanched in stainless steel kettles filled with a boiling solution of 0.1% citric acid and 1% common salt. The blanching time ranges from 5-6 minutes at 95-100°C. The mushrooms after blanching are filled in sterilized tin cans (A-2½ and A-1 tall can sizes containing approximately 440 and 220 g drained mushroom weight, respectively). Brine solution (2% salt with 0.1% citric acid or 100 ppm ascorbic acid/KMS) is added to the mushroom-filled cans after bringing its temperature to 90°C. After filling, the cans are exhausted by passing them in exhaust box for 10-15 minutes, so that temperature in the centre of cans reaches up to 85°C. Then the cans are sealed hermetically with double seamer and kept in side down position. After exhausting of cans, sterilization of cans is needed. Sterilization is the process of heating the cans up to 18°C to prevent the spoilage by microorganisms during storage. The cans cooled immediately after sterilization process to stop the over-cooking and to prevent stack burning. Cooling can be done by placing the cans in a cold-water tank. Thereafter the clean and dry cans are labeled manually or mechanically and packed in strong wooden crates or corrugated cardboard cartons. The cans are stored in cool and dry place before dispatching to market. In a hot country like India, where the ambient temperature is high during the several months in a year, basement stores are useful, especially during the summer months.

The canning unit will serve the farmers to can their unsold products and will help to avoid postharvest losses. This canning unit can be used to can the other vegetable/fruit products whenever not in use for canning of mushrooms.

The present project proposes to adopt the modern technology of mushroom processing with necessary mechanization and automation owing mainly due to large size of the project and handling of the raw materials in bulk on regular basis to achieve uniform and constant production. This shall cut down the cost of production and improve the quality of mushrooms. Low cost of production will boost competitiveness in the national and international market.

Plant and Machinery requirements

S.No.	Item
1	Freeze Dryer 1000 kg/day
2	Shorting/ Grading Line
3	Packaging Line
4	Transformer 315 KVA
5	Genset 200 KVA
6	Plastic Crates
7	Weight Scale
8	Reefer Van (4MT) - 2
9	Primary/ Minimal Processing Unit
i	Steam Generation Boiler with all accessories -1
ii	Mushroom Washer with Elevator -1
iii	Screw Blancher -1
iv	Mushroom Cooler -1
v	Mushroom Grader with Trolleys -1

vi	Mushroom Slicer -1
vii	Double Jacketed Kettle 5
viii	Exhaust Box - 2
ix	Can Seamer - 2
x	Canning Retort -4
xi	S.S. Working Table -3
xii	Cutter -3
xiii	Pulper -9
10	Quality Control Lab
11	Hygiene Component
i	Air Curtain with PVC Strip-8
ii	Flying Insect Killer-6
iii	Hand Dryer 4
12	Cold Room (30MT)-1
13	Solar System

Processed Mushroom Products

The focus of Indian mushroom industry is predominantly on trade of the fresh produce rather than the real value addition. Almost entire domestic trade is in the fresh form while most of the export is in preserved form (canned or steeped). In canning of button mushroom, 6 minutes blanching gave the best results and appropriate drained weight was attained in the cans. Pre-washing treatment with 0.05% KMS + 0.1% citric acid was found best for reducing the canning loss and retention of colour.

Various value added products such as mushrooms pickle, jam, sauce, candy, preserve, chips etc. can be prepared from fresh mushrooms whereas from the dried mushroom powder value added products like instant soup mix, bakery products, papad, nuggets etc. may be prepared. Mushroom based fortified convenience foods such as mushroom fortified noodles and mushroom based vegetarian sausages were developed using oyster mushroom. Value added products like mushroom fortified corn extrudates, fortified cakes, ready to frozen mushroom tikki were also developed. These value added products not only help to extend the shelf life of mushroom and increasing the availability for longer duration but also increase the socio-economic conditions of the growers of getting better returns. Many such value added products of mushrooms developed by post-harvest section of ICAR-Directorate of Mushroom Research (Solan) have been discussed as below:



1. Mushroom Pickle

Pickling of mushroom was found a viable economic proposition and may help in solving the glut problem during the winters. Pickling of mushrooms is an easy home scale process for preservation of mushrooms to a value added product of high market acceptability. For preparing mushroom pickle, mushrooms are washed, sliced and blanched for 5 min in 0.05% KMS solution. Blanching of white button mushroom for 6 minutes in 0.1% citric acid and 0.05% KMS resulted in better retention of whiteness of mushrooms in pickle. The blanched mushrooms are washed in cold water for 2-3 times and the excess water is drained off. Then the mushrooms are subjected to salt curing process, in which 6-7% sodium chloride is added and kept overnight. The excess water oozed-out of mushroom is removed on the next day and spices & preservatives are mixed to the desired taste and quality of mushroom pickle. To 1 kg mushroom (blanched) various spices *viz.* turmeric powder (20g), black mustard seed powder (35g), red chilli powder (10g), cumin seed powder (1.5g), carom seed (10g), nigella seed (kalonji) (10g), fennel seed powder (1.5g) and mustard oil (150 ml) are added to prepare tasty pickle. Acetic acid (5-6 ml) and sodium benzoate (0.065%) within the permitted limits are used as preservatives. This pickle can be stored up to one year in the airtight bottles. The pickle was also prepared from dried oyster mushroom and it was found that soaking in plain water for 4 hours gave the best pickle compared to soaking in water at 80°C for 5 minutes. In the similar attempt from dried button mushroom the pickle prepared was not found acceptable having good quality without any spoilage.



2. Mushroom sauce or ketch-up

Freshly harvested button mushrooms are washed, sliced and cooked in 50% of water for 20 minutes. Mushroom paste is prepared using a mixer grinder. Then salt (10%), sugar (25%), acetic acid (1.5%), sodium benzoate (0.065%), onion (10%), garlic (0.5%), ginger (3%), cumin (1 %), black pepper (0.1%), red chilli powder (1%) and arrarote (0.2%) are mixed in the paste and cooked to bring its TSS to 35 °Brix. Then the ketch-up is filled in the sterilized bottles or jars which can be stored for 6 months without any change in the quality of the product.



3. Mushroom preserve (Murabba)

For preparing mushroom preserve, fresh button mushrooms are graded, washed, pricked and blanched in 0.05% KMS solution for 10 min. Blanched mushroom is then dipped in 50 °Brix sugar solution and refrigerated overnight. Next day mushroom is strained out of sugar solution and the solution is added with 0.1% citric acid and sufficient sugar to attain strength of 60 °Brix by heating. Mushrooms are then dipped into it and kept overnight. This process is repeated to raise the concentration of syrup to 70 °Brix and mushrooms are dipped into it for 1 week to prepare preserve. The preserve is then drained out of sugar syrup and filled in a container with freshly prepared sugar syrup of 68 °Brix. The containers are then sealed airtight and stored at ambient conditions. Lug bottles were found suitable for



the mushroom preserve. The prepared preserve has a shelf life of 6 months when sealed but have to be used within 15 days after opening the container.

4. Mushroom candy

The process for making candy is practically the same as that employed in the case of mushroom preserve, with the difference that the produce is impregnated with a higher concentration of sugar (75°Brix) and is also partially dried under shade to attain the chewable consistency. The mushroom candy can be stored up to 8 months at ambient conditions with excellent acceptability. In the mushroom candy prepared, PP or PE packaging was not found suitable whereas laminates were suitable from maintained the crispness of candy.



5. Mushroom chips

Mushroom chips can be prepared from button or oyster mushroom both. For preparing mushroom chips, freshly harvested mushrooms are washed, sliced (in case of button mushrooms), divided in individual mushrooms from the bunch (in case of oyster mushrooms) and blanched in 2% brine solution. The mushrooms are dipped overnight in a solution of 0.1% of citric acid + 1.5% of NaCl + 0.3% of red chilli powder. After draining off the solution, the mushrooms are subjected to drying in cabinet dryer at 60°C for 8 h. Then it is fried in the refined oil and good quality chips are prepared. After spices mixing, the chips are packed in polypropylene (PP) packets and sealed after proper labelling. The prepared chips have to be used within three months without losing the acceptability.



6. Mushroom jam

Development of mushroom jam would aid in preserving mushrooms for a year as a product that is nutritious as well as widely acceptable. For preparation of mushroom jam, washed and blanched mushrooms are ground into a paste. This mushroom paste is then added with sugar (1:1 to paste), pectin (1% of pulp) and citric acid (1% of pulp) and heated with continuous stirring to avoid sticking to pan till it reaches a TSS of 68°Brix. This prepared jam is hot filled in sterilized glass bottles leaving a head space of 0.8 to 1.0 cm. The bottles are then sealed and stored in a cool and dry place.



7. Mushroom Biscuit

Mushroom biscuits using mushroom powder, *maida*, sugar, oil, baking powder, ammonium bicarbonate, salt, vanilla, milk powder and glucose were prepared and 8% mushroom powder was found optimum for good quality product. Both button or oyster mushroom can be used to prepare delicious and nutritious mushroom biscuits using ingredients *viz.*, refined wheat flour (*maida*) & mushroom powder (in 80:20 ratio), sugar (30%), ghee (bakery fats) (45%), baking powder (0.6 %), ammonium bicarbonate (0.3%), salt (0.6 %), milk powder (1.5 %) and vanilla essence (0.02%). For making biscuits all the dry ingredients are finely ground and sieved. Then fat and sugar are mixed well for 5-7 minutes using dough kneeder. These



ingredients are then added to dough kneeder with other dry ingredients for dry mixing of 20-25 minutes. Thereafter, water is added to make dough cohesive and homogenous and mixing is continued for 10-15 minutes. Then dough is kept for 10 minutes covered with wet cloth. Thin sheets of dough (1.25 cm thick) are made and cut into different shapes of biscuits using different steel dies. These raw cut biscuits are then baked in hot oven (at 180°C) for 20 minutes and after cooling biscuits are ready for packaging. In good quality packaging material in air tight containers, these biscuits can be stored for 3-4 months without any detrimental effect.

8. Mushroom nuggets

Among different combination 10% mushroom powder and 80% urd dal powder with other ingredients gave best mushroom nuggets. Similarly, 10% mushroom powder and 80% Masoor dal with other ingredients was also rated the best mushroom nuggets. For preparation of mushroom nuggets, mushroom powder (dried and coarsely ground mushrooms) is mixed with the black gram (*Urad*) dhal powder (1:8) and a paste is prepared by adding required quantity of water. Salt (2%) and red chilli powder (1%) are added to the prepared paste and round balls of 2-4 cm diameters are made. The prepared balls are spread over a steel tray and are sun dried. These mushroom nuggets can be straightaway deep fried and used as snacks or can be used in vegetable curry preparation.



9. Mushroom papad

Papad is a thin, crisp disc-shaped Indian snack food usually made from seasoned batter of peeled black gram flour (urd flour), lentils, chickpeas, rice, tapioca or potato, fried or cooked with dry heat. Papads can be supplemented for protein with mushroom either in the form of paste or dried powder in the batter prepared from other sources as mentioned above. This can make papad a wholesome food with high protein content. The prepared papad is packed in PP bags, sealed and stored at cool and dry place for 6 months.



10. Mushroom bhujia

Bhujia is a deep fried snack of Indian origin prepared usually from bengal gram flour (*besan*) adding salt, spices, oil and baking powder into it. Mushroom powder can be incorporated into the bengal gram flour flour up to a level to 30% to prepare nutritious and healthy mushroom bhujia. After preparation the product is sealed in air tight packets, sealed and stored at cool and dry place for 3 months.



11. Mushroom soup mix

Mushroom soup powder having mushroom powder, corn powder, milk powder, refined oil, cumin powder, black pepper and salt was prepared and found with excellent acceptability. Mushroom soup powder having mushroom powder (8%), onion powder (4%), garlic powder (4%), ginger powder (4%) and other ingredients was found the best. Mushroom soup mix has developed with oyster mushroom powder (30%), corn flour (30%), milk powder (25%), salt (8%), sugar (3%), black pepper (2%), and oregano (2%). This soup mix has to be boiled for 2 minutes with 14 times quantity of water for the preparation of good quality mushroom soup with characteristic aroma and taste. This mushroom soup mix can be stored for 90 days



at ambient temperature (25°C) and for 180 days at refrigerated temperature (4-6°C) without any significant change in sensory, proximate, vitamin D, antioxidant and microbial quality of soup mix.

12. Mushroom chutney powder

Mushroom chutney powder was also prepared from button mushroom after washing in 0.05% KMS + 0.1% citric acid, drying in cabinet drier, preparing powder and adding urd dal and other ingredients like salt, onion powder, garlic powder, ginger powder, black pepper, citric acid & red chilli powder. Among various treatments 20% mushroom powder + 20% urd dal + other ingredients gave the best product.

13. Ready-to-Serve Mushroom Curry

In view of the growing market for the readymade / ready-to-eat food items and keeping in mind the popularity of the Indian 'Curry' world over, a technology was developed at ICAR-DMR, Solan (H.P.) for production of "*Mushroom curry in flexible-retortable pouches*". The retort pouch of 105 µ thick with polypropylene outer layer (80 µ), aluminium middle layer (12.5 µ) and polyester inner layer (12.5 µ) available in the market was used for packing mushroom curry. In a frying pan, oil was added and heated. Sliced onions and green chillies were added to the oil and fried till golden brown. Garlic and ginger were ground into a paste, added and lightly fried till oil reappeared. Curry powder, salt and red chilli powder were added and lightly fried. About one litre of water was added to the spices mixture and boiled till thick consistency was obtained. Hundred grams of cut mushrooms were filled in the retort pouch and 50 g of curry was added into the pouch. Then it was heat processed for F0 value of 10 (final 13.2) at 121°C for 43 min and cooled rapidly.

The ready-to-serve mushroom curry prepared was delicious with good taste, attractive colour and a storage life of one year. Mushroom curry was also successfully prepared from dried oyster and button mushroom after its rehydration.



Novel value added products of mushrooms

Apart from various products from fresh and dried mushroom other novel mushroom based new products and fortified mushroom products have also been developed including mushroom fortified corn extrudates, mushroom fortified cakes, ready to cook frozen mushroom tikki, mushroom fortified noodles, mushroom based vegetarian sausages, mushroom snack bar, mushroom multigrain bread and mushroom health drink powder etc.

1. Mushroom fortified corn extrudates

Fortification levels of mushroom in extrudates were optimized for sensory and nutritional properties to a level of 20% paste and 10% mushroom powder for both single and twin screw extruders. Corn grits and mushroom powder or paste was blended with final moisture content of 14%. Fortification level of corn grits: mushroom powder (90:10) and corn grits: mushroom paste (80:20) was optimized according to sensory evaluation.



2. Ready to cook frozen mushroom tikki

For frozen mushroom tikki, mushrooms were used instead of potatoes to develop this novel product. Ready to cook (3 min fry) frozen mushroom tikki was developed and cohesive binding properties of mushroom shreds was optimized by using response surface methodology and taking shred size, corn starch concentration and par frying time as the variables. Optimization was done on basis of fat absorption characteristics, textural and sensory properties.

3. Mushroom based vegetarian sausages

Vegetarian sausages can be prepared from fresh mushroom by adding 5% saturated fat and binding agents such as carrageenan, soya protein concentrate, casein or xanthan gum.

4. Mushroom fortified cakes

Mushroom fortified cakes have also been developed and fortification to a level of 20% (as wheat flour replacement) was found to be optimum according to sensory and textural properties of both cake and batter prior to baking.



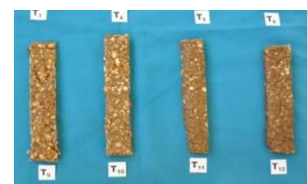
5. Mushroom fortified instant noodles

Ready to cook instant noodles fortified with graded levels of mushroom (*Pleurotus ostreatus*) powder have been developed and on the basis of their nutritional and sensory properties, the level of fortification of mushroom powder @ 4 % to noodle dough was optimized.



6. Mushroom snack bar

Mushroom fortified snack bar was developed with white button mushroom powder (20%), sweeteners (40%), cereals (25%), peanuts (10%) and dry fruits (5%). This mushroom fortified snack bar can be stored for 30 days at ambient temperature and for 90 days at refrigerated temperature without any significant changes in sensory, proximate, vitamin D, antioxidant and microbial quality of snack bar.



7. Mushroom multigrain bread

Mushroom fortified multigrain bread was developed with oyster mushroom powder (5%), refined wheat flour (85%), whole wheat flour (6%), ragi flour (2%), oatmeal (2%), and flaxseeds (1%). This bread was acceptable based on sensory analysis (overall acceptability score-6.88) and contained 8.23% protein, 30.54% carbohydrate, 1.36% fat, 1.02% ash, 0.21% fiber, 1264.72 IU vitamin D, 4.07mg/100g iron, 198.44mg/100g calcium, 9.29 manganese, 1.3 copper, 1.27 zinc, 32.93 potassium and good antioxidant-properties (DPPH- 88.18 mg AEAC/100g, ABTS- 489.24 mg AEAC/100g). Shelf life of this mushroom fortified multigrain bread was determined to be 5 days at ambient temperature without any significant change in sensory, proximate, vitamin D, antioxidant and microbial quality of bread.



8. Mushroom health drink powder

Mushroom fortified health drink powder was developed with oyster mushroom powder (10%), malted ragi (10%), whey-protein (20%), milk powder (30%), sugar (20%) and cocoa (10%). This health drink powder was found to be acceptable based on overall-acceptability score (8.17) and contained 25.01% protein, 62.55% carbohydrate, 3.96% ash, 6.43% fat, 4.53% fiber, 2435.9IU vitamin D and good antioxidant-properties (DPPH-394.75 mg AEAC/100g, ABTS- 1055.98 mg AEAC/100g). The product has a shelf life of 6 months at cool and dry place.



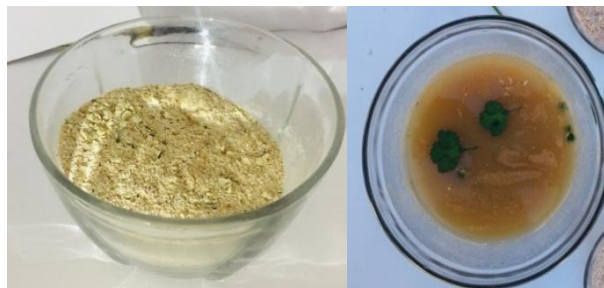
9. Oyster mushroom spread

A nutritious and tasty mushroom spread was developed using oyster mushroom (10g) and tomato (90g) along with garlic (2g), ginger (1g), chilli (1g), salt (1g), sugar (1g), vinegar (2ml), vegetable oil (2ml), black pepper (0.5g) and oregano (0.5g). This spread can be used with bread, sandwiches, burgers, pizza, etc. The developed spread was found acceptable based on sensory analysis and contained 70.03% moisture, 2.97% protein, 14.77% carbohydrate, 7.78% fat, 4.44% ash, 2.45% fiber and 240.07 IU/g vitamin D. This spread has a shelf life of three months at room temperature.



10. Shiitake mushroom vegetables soup mix

A mushroom vegetable mixed soup mix was developed using shiitake mushroom powder (20%) along with vegetables mix (containing tomato powder, dried carrot shreds, partially cooked and dried peas, onion powder and garlic powder) (15%), corn flour (27.5%), milk powder (22.5%), salt (9%), sugar (3%), black pepper (2%) and oregano (1%). The developed soup mix was found acceptable based on sensory analysis and contained 2.8 % moisture, 8.62% protein, 71.44% carbohydrate, 4.02% fat, 13.12% ash, 3.47% fibre and 2681.48 IU/g vitamin D. The prepared products can be stored for three months at cool and dry place.



GHP/GMP/HACCP in mushrooms

Good Manufacturing Practices (GMP) /Good Hygiene Practices (GHP) - All practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain

Good Manufacturing Practices (GMP) – manufacture & process controls & includes supplier control; specifications; calibration of equipment; traceability & recall; equipment designs where conditions for food safety can be achieved, maintained & monitored; lighting & ventilation systems; storage conditions; control of operations

Good Hygiene Practices (GHP) – system/ measures for maintaining hygiene & sanitation include personal hygiene & employee health conditions, maintenance of plant & equipment hygiene including food contact surfaces, pest control, waste disposal, water quality, toilet & hand wash facilities, prevention of cross contamination

Hazar Analysis Critical Control Points (HACCP) is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product.

HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards based on the following seven principles:

Principle 1: Conduct a hazard analysis.

Principle 2: Determine the critical control points (CCPs).

Principle 3: Establish critical limits.

Principle 4: Establish monitoring procedures.

Principle 5: Establish corrective actions.

Principle 6: Establish verification procedures.

Principle 7: Establish record-keeping and documentation procedures.

HACCP for fresh mushrooms

The reception stage of mushrooms is a critical control point (CCP). The main hazards at this stage are: the presence of unauthorized phyto-sanitary products; larger doses of such products than those permitted; the presence of pathogenic bacteria or thermo-stable entero-toxins.

HACCP for dehydrated mushrooms

CCPs for mushroom dehydration would be; the water used for washing the mushroom, dehydration temperature and packaging process and metal detection

HACCP for Mushroom pickle

Critical control points in the whole process of making mushroom pickles are water used for washing the mushroom, blanching temperature, pH of pickle and metal detection.

HACCP for mushroom based bakery products

Critical control points in making mushroom biscuits, bread or cake would be moisture content in mushroom powder, raw materials quality, baking temperature, packaging and metal detection.

HACCP for Mushroom chips

5 CCPs that has potential hazard for making mushroom chips would be: the water used for washing the mushroom, the boiling process, the frying process, the chips draining process, and the packaging process.